BIRD MALARIA

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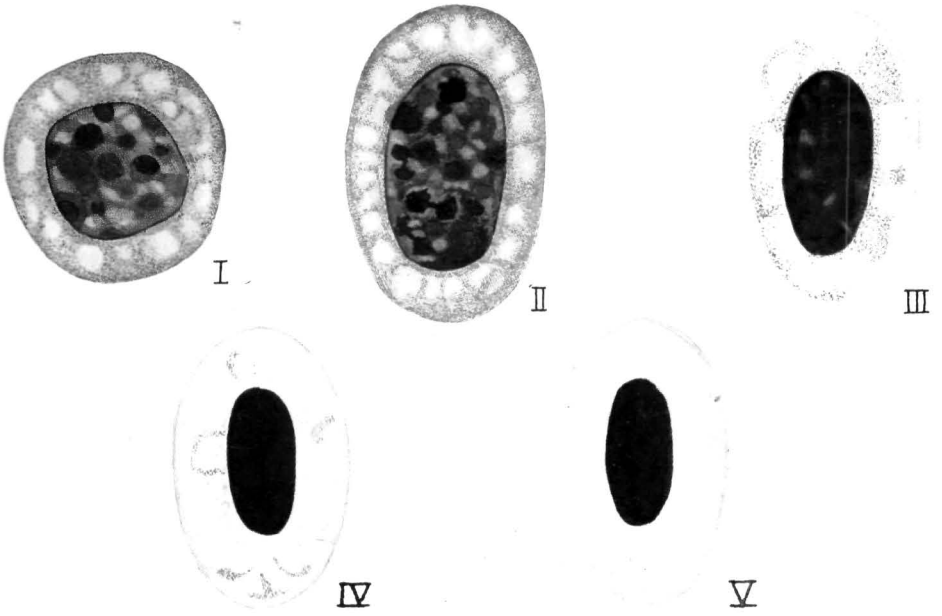
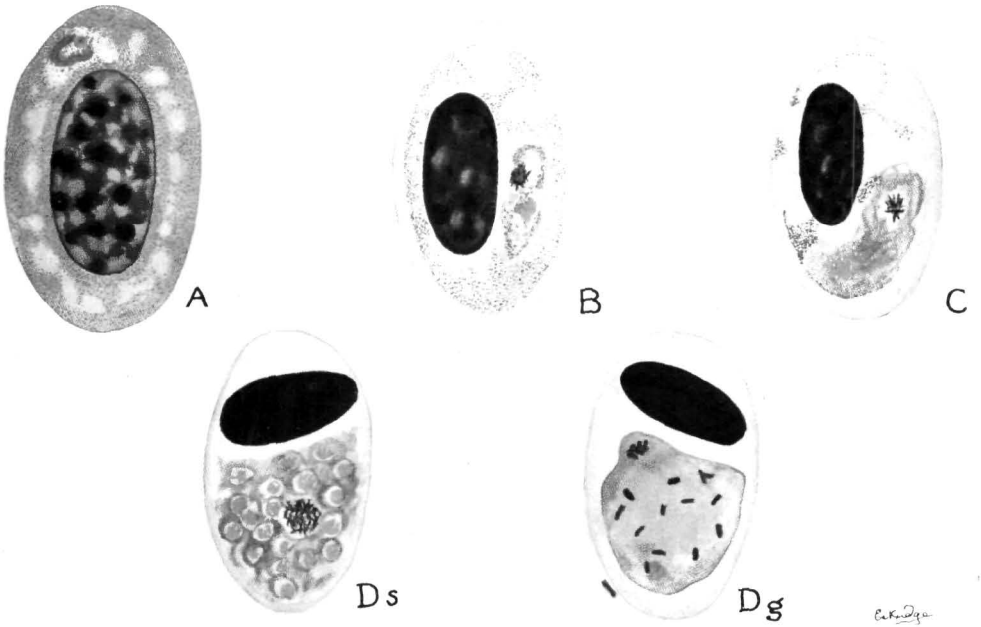


TYPES OF RED BLOOD CELLS (CANARY)



TYPES OF PARASITES (P. CATHEMERIUM)

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TO

ROBERT HEGNER AND REGINALD D. MANWELL

Who for many years have encouraged research on bird malaria in the United States, and have themselves made many lasting contributions to the field.

PREFACE

In the past fifteen years investigations on the malaria parasites of birds have been one of the most productive phases of experimental research in the field of protozoan parasites throughout the world. More than two-thirds of the literature on the subject, since the discovery of avian plasmodia by Danilewsky in 1885, has appeared within this period. Although graduate students and research workers who have been interested in certain specialized aspects of bird malaria have been able to obtain brief reviews of some of the literature in summary papers or text books of various sorts, no one source has been available where information relative to the materials, methods and results in the entire field could be obtained. The purpose of this monograph is an attempt to fill this need.

The plan at first was to present abstracts of all the published literature in chronological order, but it soon became apparent that such a presentation would be of little value to beginning students or to advanced workers who were not familiar with the early history of the subject. It was also found that to include detailed discussions of every paper in the field would add too greatly to the cost of publication, and would probably prohibit it altogether. A certain amount of selection has therefore been made in the choice of subject material included in the various chapters and it is hoped that omissions will not be regarded as intentional oversights. References to *Haemoproteus* are included only when they have a direct bearing on the subject under discussion.

Figures and tables have been taken freely from the work of various authors and these are acknowledged herein. Special thanks are expressed to Dr. Robert Hegner for encouragement, suggestions and criticism throughout the preparation of the text. Permission to reproduce plate IX and table 9 was kindly given by Dr. Fruma Wolfson, and Mr. Marion Brooke helped in the preparation of some of the photo-

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REDGINAL HEWITT.

Baltimore, Maryland,
1940.

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INTRODUCTION

To estimate the full part that research on bird malaria has played in the development of knowledge of malaria parasites in general during the past fifty-five years is a task which cannot be fully appreciated until the general aspects of the field in its entirety have been surveyed. It is fortunate that plasmodia in birds were found (Danilewsky, 1885) so soon after Laveran (1880) described the causative organisms of human malaria, since the course of experimental observations with both types of parasites has proceeded side by side from the time of their discovery. Without the one, knowledge of the other would probably never have reached the position of importance in the field of medical zoology that both now hold. In 1891 Laveran made the following statement which explains the early interest which medical men as well as zoologists had in the field of avian malariology: " Direct observations on the parasites of human malaria appear to have reached the point where we can expect little more from them, and I believe that in order to solve the now obscure questions relative to the evolution of these parasites, it is necessary to study the analogous parasites which exist in animals other than man. The blood parasites of birds described by Danilewsky present a particular interest in this connection, because of their great resemblance to the human malaria parasites. It is here that medical men can encroach upon the domain of the naturalists and occupy themselves with the study of blood parasites of birds."

Subsequent research has borne out this prophecy, although recent developments through the use of human malaria parasites for the treatment of paresis, and newer knowledge concerning species and methods involved in work on monkey malaria have greatly widened the experimental approach to the practical problems of human malaria.

In the early researches on the plasmodia of birds the underlying impetus was to discover facts that could be directly

applied to human malaria, and would therefore augment existing knowledge concerning the morphology and physiology of human malaria parasites. The important work of MacCallum (1897 and 1898) on the fertilization of the female gamete, followed by Ross's (1898) discovery of the mosquito transmission of malaria through the use of birds as experimental hosts were the first and will probably remain the most prominent contributions in the field as a whole. Roehl's (1926) discovery of plasmochin and Kikuth's (1932) work on atebrin introduced two new synthetic malaricidal drugs which have since been widely adopted in the treatment of human malaria. These compounds were first tested on the plasmodia of birds.

For the past twenty years the Department of Protozoology of the School of Hygiene and Public Health, the Johns Hopkins University, under the direction of Dr. Robert Hegner, has been actively engaged in research on bird malaria. This work was begun in 1920 and the first paper published was that of Ben-Harel (1923) on the characteristics of infections, which included parasite counts, blood counts, and a study of pathology and relapse phenomena. The first strain of bird malaria used in this laboratory was obtained from Dr. Whitmore, who isolated it from a sparrow caught in New York in 1913 and carried it in canaries at the Army Medical School. G. H. Boyd began a study of the course of infections, drug therapy and host modifications with *Plasmodium relictum* in 1923, 1924 and 1925. This work was followed by that of Hartman (1925) and L. G. Taliaferro (1925) on studies of the asexual cycle, rate of reproduction, periodicity and species differentiation. Drensky and Hegner (1926) confirmed Mrs. Taliaferro's findings of a 24 hour periodicity in *P. cathemerium.* Hegner and MacDougall (1926) and MacDougall (1927) studied the effects of sugar and insulin on *P. cathemerium* infections, and from 1923 to 1928 the International Health Division of the Rockefeller Foundation supported a program on the effect of various types of drugs on avian plasmodia. Dr. Hegner conducted this work with the assistance of G. H. Boyd, Hartman, Shaw, Luengo, Val-

labhakorn, Gingrich, and Manwell. In 1927 Huff began his work on the mosquito transmission of bird malaria, including problems involving the morphology of oöcysts and sporozoites and the natural immunity of certain mosquitoes to infection. Wampler (1928) studied the effects of quinine and plasmochin on *P. cathemerium.* Shah (1934), Rozeboom and Shah (1934), and Shah, Rozeboom and Del Rosario (1934) studied the sexual stages in the vertebrate host and in the mosquito. Since 1934, Wolfson has published papers on the periodicity of various species of avian plasmodia, on a species from the owl, on the reaction of the parasites in abnormal hosts, and on exoerythrocytic parasites found in association with *P. cathemerium* and *P. relictum.* Young (1937 and 1938) studied the pathology of *P. rouxi* infections, and Herman (1937, 1938 and 1939) published on the incidence of malaria in wild birds and the epidemiology of infections in nature. Hegner and Hewitt (1937 and 1938), Hewitt (1938 and 1939) and Hegner and Eskridge (1938) have described the penetration of young red cells by four species of avian plasmodia. Hegner and Wolfson (1939 and 1940) have conducted experiments on exoerythrocytic parasites, Hegner and Eskridge (1939) on passive immunity and the mortality of merozoites, Hegner and Dobler (1939) on passive immunity, and Hegner and West (in press) on the behavior of plasmodia in abnormal hosts. Hewitt (1940) studied the malaria parasites of Mexican birds. Grants were made in 1938 and 1939 by the Rockefeller Foundation and by the John and Mary Markle Foundation for studies on the penetration of young red cells by merozoites and cytological studies on the parasites. This work is being carried out by Dr. Sidney Velick and Dr. T. T. Chen, but at the time of this writing has not been completed. A study of *P. lophurae* in the chicken has been made by Terzian (in press).

Several of the workers mentioned above have been called to other universities in the United States and have there continued research programs on bird malaria. Among these may be mentioned W. H. and L. G. Taliaferro, and Huff (Uni-

versity of Chicago), Manwell (Syracuse University), and G. H. Boyd (University of Georgia).

The development of research on bird malaria in European laboratories has progressed in a similar fashion. Many problems have been studied by the Sergent brothers and their co-workers at the Pasteur Institute in Algiers, Brumpt and his co-workers in France, Kikuth in Germany, and Missiroli, Raffaele, Corradetti, Giovannola and others in Italy.

A criticism which has been expressed with regard to the practical application of results obtained from research on bird malaria is that the avian plasmodia probably differ in many respects from human malaria parasites in their morphology, physiology, and reactions to host modifications. To a certain extent, this is undoubtedly true, but as new research on therapeutic malaria and monkey malaria appears, it becomes increasingly apparent that much of the experimental work which has been done with birds can be directly repeated with mammalian plasmodia. For example, Young, Coatney and Stubbs (unpublished at the time of this writing) find that the periodicity of segmentation in a *P. malariae* infection may be reversed by changing the host's period of rest and activity. This is exactly comparable to the work of G. H. Boyd (1929), Stauber (1937, 1939) and others on *P. cathemerium* and *P. relictum* in birds. The effects of drugs on bird malaria parasites gives a fairly reliable index as to what will happen when the same drugs are given to human beings suffering from malaria. Much of the information concerning the mechanism of immunity in malaria, particularly the cellular reactions, has been derived from experiments on birds, since the only possible way to determine the nature of some of these phenomena is to kill the experimental host at the proper period during infections. Cannon and Taliaferro's work (1931) on *P. cathemerium* in this connection is very similar to the results that the same authors (1936) obtained with *P. brazilianum* infections in monkeys. Mark Boyd's investigations on species and strain immunity in human malaria is paralleled by the reciprocal strain immunity and species immunity exhibited by all

of the avian plasmodia which have thus far been studied. Many other similar examples might be cited, and there is little reason for believing that the types of infections produced in bird malaria, monkey malaria, or human malaria are essentially different. Since the cost of maintaining facilities for experimental work on birds is far less than that involved in the laboratory study of monkey malaria or human malaria, studies on bird malaria will continue to supply much helpful research material.

The use of large experimental birds for laboratory hosts within the past five years has greatly increased the opportunities for research on bird malaria. *P. gallinaceum* in chickens (Brumpt, 1935) is being widely studied in European laboratories. Coggeshall (1938) isolated *P. lophurae* from a fire-back pheasant, and this species is inoculable to chicks. *P. relictum* has been found in pigeons and doves (Coatney, 1938) and Wolfson (1939) has successfully transmitted both *P. relictum* and *P. cathemerium* to ducks. In this laboratory, also, infections with *P. cathemerium* have been obtained in turkeys and chickens. Further examinations of large wild birds will probably reveal other species of *Plasmodium*, or new strains of species now known.

CHAPTER I

THE DISCOVERY AND EARLY HISTORY OF BIRD MALARIA

1. DANILEWSKY

The first mention of intracorpuscular parasites in the blood cells of birds was made by Danilewsky in 1885. Without knowledge of the description of malarial organisms in man by Laveran (1880), Danilewsky briefly but accurately described similar parasites from birds, although at the time he did not know what they were. This original paper was not alone concerned with the description of bird malaria parasites, but dealt with trypanosomes of birds, as well as with haemogregarines from lizards and tortoises. It is unfortunate that Danilewsky did not know of Laveran's discovery, because it was necessary for him to use words of his own manufacture to describe the parasites in birds, and in so doing he created a nomenclature which helped not a little to confuse the literature. However, the additions which he made to the then known list of descriptive terms for malaria organisms was by no means less warranted than some of the names suggested by workers in the field of human malaria in the early years following Laveran's first description of these parasites.

"*Pseudovacuoles.*" In Volume 5 of the "Biologisches Centralblatt" Danilewsky describes three types of parasites from the blood of birds. The first type occurred free in the plasma and appeared to him to be a type of gregarine or "little blood worm." The second blood parasite was a trypanosome, also noted free in the plasma. A third was found to be both intracorpuscular and at certain times free-swimming in the plasma; we now know this to be a species of bird malaria parasite. The following is a literal translation of Danilewsky's description of the organism:

"The third form is a haemocytozoon, which, after excapsulation, also is found free-swimming in the plasma. In the interior of red blood cells these parasites appear as clear, colorless, transparent vacuoles, variable in shape and size, in which are present several refractile glossy-black granules. These pseudovacuoles are very common in certain species of birds. They lie beside the nucleus of the blood cell and are arranged in a large circle. The more developed forms take on a globular shape, altering the outline of the blood cells, which at the same time become more and more distorted."

In the same paper he described the rupture of the host cell and the escape of the large globular bodies into the plasma, with resulting exflagellation, although at the time he had no idea of the significance of the latter.

"*La Parasitologie Compareé du Sang.*" During the ten years which followed his discovery of malaria parasites in birds, Danilewsky published several papers describing further researches, chiefly in French and German journals. It is to his monograph "La Parasitologie Compareé du Sang," published in 1889, that we are most indebted for detailed descriptions and comments on a great many phases of bird malaria infections which are now quite familiar to us but were first mentioned by him. At the time he wrote this monograph, Danilewsky had become familiar with the work of Laveran, and Marchiafava and Celli on human malaria. He approached the problem from a medical point of view, and most of the work which he reported after 1886 had direct implication to similarities between avian and human malaria.

The first part of his monograph ("Nouvelles recherches sur les parasites du sang des oiseaux") summarizes his researches up to the year 1889 on the blood parasites of birds. This had been published "*in extenso*" in Russian one year before. His purpose in writing the monograph is expressed in the preface as follows:

"I believe that these researches will throw some light on the complicated questions concerning the nature of the ma-

laria parasites of man and in so doing will enlarge and facilitate the experimental study of malaria in general."

The comparison between bird malaria and human malaria is stressed throughout the text, although he attacked certain problems from a purely biological standpoint. His experimental animals were various species of wild birds, trapped or shot in the vicinities of Kharkov, Kherson and Kouban (Southwestern Russia). When infections were found, blood examinations were made for a period of months and the course of the disease was thus followed. None of the birds were artificially inoculated. It is quite probable that the majority of infections which he found were of a chronic nature. Of 300 birds examined only 4 or 5 died from the probable effects of malaria. When autopsies were made it was noted that both the spleen and liver were greatly augmented in volume and each contained enormous amounts of black pigment. His description of malarial pathology, although brief, includes the three primary macroscopic changes in the organs of infected birds, namely, enlargement of the liver, enlargement of the spleen, and the deposition of pigment in these organs. It was further noted that the pigment in the spleen was contained within macrophages, and that these same macrophages often contained 1 or 2 blood cells in the process of disintegration.

Inasmuch as the mosquito transmission of malaria had not been discovered at the time of Danilewsky's monograph, it is of interest to note his conception of the epidemiology of bird malaria, expressed as follows:

" I have already emphasized that for four Summers I have made observations on the Haematozoa of various kinds of birds, and only members of the Class Insessores have been found to be parasitized, never members of the Class Autophagae. The first, as you know, are nursed by their parents for a certain time after hatching, e. g. receive their nourishment directly from the mouth of the latter, the others on the contrary are left to seek out their own food. If we can generalize on these facts, it appears that Hematozoa are able

to exist only in birds which receive their first nourishment directly from the mouths of their parents." (The implication here is that the malaria parasites are transmitted from parent to offspring by mouth.)

Another very interesting observation made by Danilewsky at this time was the fact that young parasites are frequently found within young red cells. He recognized that this was not a chance occurrence, and although he gives no quantitative data to support his theory, he suggested that young parasites matured along with the red cells which contained them. It occurred to him that such a relationship might take place most easily in the haemopoeitic organs, principally the bone marrow, and he attached great significance to the fact that the penetration of red cells occurs in the centers of erythrogenic activity.

The interpretation of exflagellation is one of the outstanding parts of Danilewsky's monograph. In his first paper he described somewhat confusedly the formation of flagellated bodies from " pseudovacuolae " which had escaped from the host cell when placed in mounting fluid under the microscope. This puzzled him profoundly, as it did all workers at that time who had noted the phenomenon in malarious blood from human beings. It is to be remembered also that Danilewsky knew nothing of the previous reports of these flagellated bodies by Laveran when he wrote his original description. After he had read the reports of similar occurrences in the parasites of human malaria, however, he described more fully what he had observed. The description which he gives of the escape of male gametes from the gametocyte is quite as accurate as any made by modern investigators, although, of course, he did not know that they were male gametes, and indeed was far from seeing their true significance. He believed that the large " pseudovacuole " was a kind of cyst containing the flagellated organisms. Whether or not he believed they had any sexual significance can not be determined from his writings, although it is evident that he did consider the flagellated organisms to be part of the life cycle of the malaria parasite.

Danilewsky's comparison of human and bird malaria throughout this work without doubt served to stimulate the great amount of research which followed. This collection of observations was referred to by the Italian, French and German workers who continued the study. In it are described some of the fundamental processes in the growth, life history, pathology, symptomatology, and immunology of bird malaria organisms.

Further work. Following the publication of his monograph, Danilewsky continued his researches with the malaria parasites of birds and in 1890 published three papers in the Annals of the Pasteur Institute. The first of these deals chiefly with a description of *Leucocytozoon.* In a second paper his observations on the phagocytosis of haemogregarines and malaria parasites by white blood cells are presented. A distinction is made in the third paper between acute and chronic malaria infections in birds and a general comparison is given of malaria in birds and man. Many of our modern notions with regard to host-parasite relationships are expressed in these papers, and the influence of Danilewsky's contemporaries (Metchnikoff, Grassi and Feletti) is clearly shown. The following excerpt, translated from the French, shows that his conception of the struggle between host and parasite was not considerably different in principle from the views which many malariologists now hold:

"The microscopical researches of these past years have acquainted us with many examples of intracellular parasites which inhabit the red corpuscles (*Drepanidium, Hemogregarinae, Polimitus*, etc.). It has been demonstrated by my observations that, in birds, these cytozoic parasites penetrate the blood-cell precursors, leucocytes, lymphocytes, erythroblasts and hematoblasts, in the form of small germs, and these develop parallelly with the progressive metamorphosis of the blood elements themselves. Part of these eventually succumb to the conflict between the phagocytic activity of certain cells, while others conserve their vitality and, in so

doing, are able to develop to maturity. It is very probable that the same conflict occurs in the malaria infection in man, above all in the spleen and in the bone marrow. We may consider these organs as porous filters, in which the blood leaves in passing, foreign bodies and the parasites, which are actively engulfed by the protoplasmic elements. The structure of these organs and their mechanical relation with the blood explains and confirms this interpretation. Direct observations have demonstrated that the bone marrow is generally more rich in parasites than the blood. Furthermore one can often find a large number of parasites in the bone marrow when they cannot be demonstrated in the blood. This is true not only of intracellular parasites, but also of free parasites, such as *Trypanosoma sanguinis* (in birds)."

Many erroneous convictions were held by Danilewsky and these are frequently expressed in all of his papers, but it is not easy to condemn his train of thought in the light of the little knowledge which was available to him at the start of his researches. He was convinced, for example, that the malaria parasites of birds and those of man were identical. He did realize, however, that it would be necessary to artificially infect man with malaria from birds before this point could be proved.

No further important research in the field of bird malaria was carried out by Danilewsky following the above mentioned papers. One paper in 1891 and another in 1895 dealt chiefly with the relationships between human and avian plasmodia. At this time many other famous names were being added to the charter roll of malaria research. Danilewsky's work, however, was not lost in the wealth of material that was accumulating in many of the research centers of Europe. Most of his immediate followers, but very few modern investigators, have noted and profited from his original observations and descriptions of the plasmodia of birds.

In a recent biographical sketch of Basil Danilewsky, Hoare (1939) presents some very interesting facts about his life, which, to the present writer at least, were heretofore not known. He was born in Kharkov, Russia, in 1852, and re-

ceived his medical degree from the University of Kharkov in 1877. Aside from his researches on the blood protozoa, Danilewsky's scientific and teaching career was devoted almost exclusively to physiology. He held chairs in physiology at the University of Kharkov, the Kharkov Veterinary Institute, and the Kharkov Medical Institute for Women. At the time of his death (February 25, 1939) he was head of the Ukranian Institute of Endocrinology and Organotherapy in Kharkov, and was the oldest surviving Honorary Fellow of the Royal Society of Tropical Medicine and Hygiene in London. As a scientist, Danilewsky has been apparently more widely recognized as a physiologist than as the first avian malariologist, but his brief contact with this field of medical zoology has left a very lasting impression.

2. GRASSI AND FELETTI

Following Danilewsky, Grassi and Feletti were next in importance in the early history of bird malaria. In 1890 these authors reported two distinct types of intracorpuscular parasites in sparrows (*Passer hispaniolensis*) and in pigeons. Since they were familiar with malaria parasites in human beings they realized that the bodies which they found in the red cells of birds were definitely of the same type. Grassi was a zoologist and a keen observer of morphological details. The genus *Laverania* was established to include the crescent-shaped parasites already described by Danilewsky in birds, as well as similar parasites in man described by Laveran. The parasite in birds was called *Laverania danilewsky* and in man, *Laverania malariae.* The latter name is still used by some modern investigators for what is now generally known as *Plasmodium falciparum.* It is in some ways unfortunate that *L. danilewsky* cannot be used today as the name for the parasite which is now called *Haemoproteus*, but according to the rules of priority, the name *Haemoproteus*, given to the same parasite by Kruse (1890) a short time before Grassi and Feletti's paper appeared, must be given preference.

The second parasite described by Grassi and Feletti was

smaller than the first and was regarded as closely related to the amoeboid forms of the human malaria parasite. In fact it was their belief that this parasite was identical with the similar parasite of human malaria. This has given rise to an interesting situation regarding the correct scientific name of the parasite with which they were working. They suggested the genus name *Haemamoeba* and proposed the name *Haemamoeba praecox* for both the bird malaria parasite and the human parasite. A second species was described as *Haemamoeba immaculata* because of the supposed absence of pigment.

During the course of their researches in 1890 and 1891 Grassi and Feletti observed more and more structural differences between the avian and human parasites, and in 1891 changed the name *Haemamoeba praecox* to *H. relicta*, retaining the name *H. praecox* for the parasite of human malaria. Another type of organism was described by them in 1892, similar in almost every detail to *H. relicta* but differing from it in the length of the schizogonic cycle. This seemed to them to be a fairly constant feature, so they gave the name *H. subpraecox* to the organism in which schizogony began before it occupied a large part of the red cell. A third type of organism, devoid of pigment, was called *H. subimmaculata*, in order to distinguish it from *H. immaculata* which had been previously described from man as well as from birds. This shifting of specific names in an attempt to differentiate human and bird malaria resulted in a tremendous amount of confusion, which to this day has not been entirely cleared up to the satisfaction of all malariologists. Furthermore, the names *Haemamoeba praecox* and *Laverania malariae* were both assigned to the organism which produces malignant tertian fever in man. However, the fact that Grassi and Feletti put the parasite which they found in birds in the same genus with the intracorpuscular parasites of man was a contribution of importance. Their familiarity with the work of Laveran gave them an advantage over Danilewsky, since the latter was at loss to know how to classify the organisms which he had discovered.

As in the case of Danilewsky, the interest of Grassi and Feletti quickly shifted to the study of human malaria, and after 1892 no further important work with bird malaria appeared under their names. Their chief contribution, other than confirming Danilewsky's work, was that of distinguishing avian plasmodia on definite morphological characteristics. They added considerably to the descriptions of the parasite given by Danilewsky and no doubt stimulated further morphological work.

3. KRUSE AND PFEIFFER

Both of these men reviewed the existing knowledge of intracorpuscular parasites, and suggested a nomenclature for all of the types which had been described. Neither, however, presented much original research material. Kruse (1890) is best known for establishing *Haemoproteus* as a generic name for the crescent-shaped bodies found by Danilewsky in the blood of birds. Pfeiffer (1890) held to the nomenclature of Grassi and Feletti in classifying the malaria parasites, recognizing four species of *Haemamoeba* in man and three in birds. He accepted the genus *Laverania* for the crescent-shaped intracorpuscular parasites of man, birds, and frogs.

4. LAVERAN

It is of interest that the discoverer of malaria parasites in man should play a part in the early researches on bird malaria. Laveran's first publication on bird malaria appeared in 1890 entitled " Des hématozoaires viosins de ceux du paludisme observes chez les oiseaux." In this paper he discusses Danilewsky's descriptions of blood parasites from shrikes, jays and owls, and states that his own observations confirm those of Danilēwsky. Laveran was eager to find in such a convenient laboratory host as the bird a solution of some of the puzzling problems which he, as well as others, encountered in studies on human malaria. His first paper was merely a redescription of the morphology of the bird malaria parasite with notes on pathological lesions. He mentions

that in June, 1889, he had tried to infect a jay with malarial blood from another jay without success. In 1891 he published two papers on the general characteristics of malaria parasites in birds, and in the same year presented photomicrographs of both bird malaria and human malaria to the Academy of Sciences in Paris. His experimental animals at this time were chaffinches, jays and larks, but he mentions that he would like to have had a larger bird with which to carry out his researches.

Laveran's next paper (1893) was a sharp rebuttal of some of Alfonse Labbé's remarks to the Societé de Biologie with reference to " la forme spherique des hematozoaires " (gametocytes) in birds and man. The dissention between Laveran and Labbé was probably stimulated by the bitterness which Labbé held towards medical men engaged in research. Although Labbé's career in the field of bird malaria was short he took several opportunities to thrust at some of the theoretical considerations proposed by Laveran and presented by him to the Societé. This difference of opinion was revealed in several papers in 1893. Labbé believed that the flagellated bodies, which were formed when malarial blood was removed from birds and placed in isotonic saline under the microscope, were definitely not degenerating or abnormal bodies, but were the result of some physicochemical change brought about by the removal of the blood from the host. He furthermore stated that they were rarely if ever found in circulating blood. Laveran, on the other hand, declared that these bodies could frequently be found in circulating or freshly-drawn blood and that Labbé's explanation could not be accepted. The end result of the argument was that both men retained their original views.

In succeeding years Laveran published a number of papers on bird malaria, most of these appearing in the " Comptes Rendus de la Societé Biologie." A malaria parasite (*P. majoris*) from Java sparrows was described by him in 1898 and was elaborated upon in a later paper (1900). In two papers (1899, 1901) he prepared a classification of the then known malaria parasites. Further miscellaneous papers on morph-

ology (1901), staining techniques (1902, 1903), and host records (1902, 1905, 1914) were published by him, singly and in joint authorship.

Laveran's contribution to our knowledge of bird malaria was perhaps not so great as that of Danilewsky or Labbé, but, above all else, he was responsible for stimulating medical men to seek the answer to many of their malaria problems with the aid of the plasmodia of birds.

5. CELLI AND SAN FELICE

Following Grassi and Feletti, the two most important Italian workers in the early studies on bird malaria were Celli and San Felice (1891). Comparatively little material on avian parasites was published by these workers but their attempts to classify intracorpuscular parasites in birds give them a prominent place in the early literature on the subject. They expressed the view that three principle types of malaria parasites live in birds: (a) parasites similar to the crescent-shaped organisms described by Danilewsky and others, which they thought to be analagous to the quartan parasite of man; (b) parasites with a shorter period of development than the above; and (c) parasites with a still shorter period of development, which produced fewer merozoites than the second type. It is believed that these workers were dealing with mixed infections of *Plasmodium* and *Haemoproteus.* No attempt was made to assign definite developmental cycles to any of the parasites described.

A more concrete contribution by Celli and San Felice was the first successful transfer of malaria parasites from bird to bird by blood inoculation. This had been tried without success by nearly all preceding workers, probably due to the fact that most of them used *Haemoproteus* in their inoculation experiments; since only gametocytes of this genus are found in the peripheral blood it cannot be transferred from bird to bird by blood inoculation.

Celli and San Felice believed that the malaria parasites of man and those of birds were similar but not necessarily identical. In various species of birds they sometimes found

two types of intracorpuscular parasites; these they thought to be polymorphic stages of a single species. They also believed that each species of bird harbored its own variety of parasite and that this variety could not be inoculated into other species of birds.

6. LABBÉ

If the work of Alfonse Labbé and that of Danilewsky were our sole sources of knowledge of bird malaria we would still be well informed on the subject. Labbé's publications were not numerous but contained much sound material. As mentioned earlier, his first publication on bird malaria culminated in a series of arguments with Laveran on the nature of the flagellated bodies which frequently occurred in drawn blood. At that time Labbé was working towards the Doctor of Science degree at the Sorbonne in Paris. In the same year (1893) he described blood-cell parasites from larks. As in the case of Danilewsky, however, these short preliminary papers were followed by a much more important piece of work, a monograph of 208 pages (1894). This publication was Labbé's thesis, presented to the faculty of sciences of Paris.

Labbé was not a physician and attacked the study of blood-cell parasites from a strictly zoological point of view. In fact, as mentioned earlier, he did not hesitate to express his belief that the medical doctors of his time were for the most part poor zoologists. This seemed to him to be particularly true in the case of work done on the blood parasites, naturally enough, since this was his own interest.

The first part of his monograph consists of a review of the then-known systems of classifying blood parasites that had been suggested by Danilewsky, Grassi and Feletti, Kruse, and Pfeiffer. He then outlines his own classification and suggests the generic name *Proteosoma* for the parasites which Danilewsky had described under the name *Cytosporon* and Grassi and Feletti under the name *Haemamoeba.* Labbé likewise introduced the name *Halteridium* for Kruse's *Haemoproteus.* He was perhaps justified in adding two new

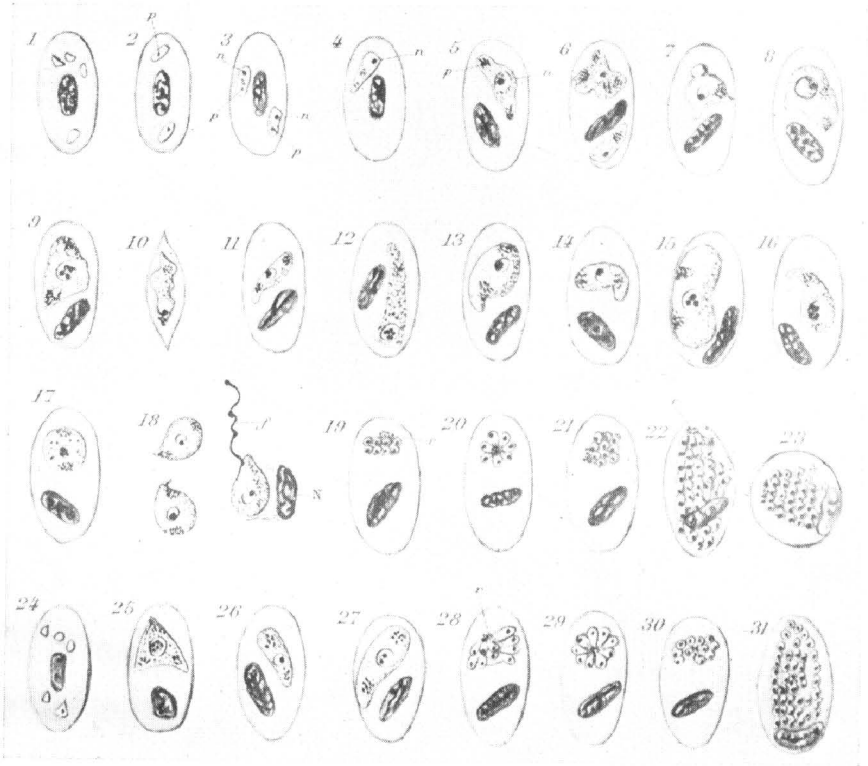


PLATE II—A photograph of Labbé's drawings (1894) of bird malaria parasites.

Figures 1-23. *Proteosoma* (*Plasmodium*) in the chaffinch. 1. Small non-pigmented forms. 2. Small pigmented forms. 3. Young trophozoites. 4-10. Amoeboid forms. 11-16. "Gregarine-like" forms. 19-21. Early sporulating forms. 22-23. Later sporulating forms.

Figures 24-31. *Proteosoma* (*Plasmodium*) in the lark. 24. Young trophozoites. 25. Amoeboid form. 26-27. "Gregarine-like" forms. 28-30. Early sporulating forms. 31. Later sporulating form.

names to the already much abused nomenclature for blood parasites, because two or more species, and perhaps even genera, were included under the names *Haemamoeba* and *Haemoproteus.* The name *Proteosoma* was widely used for the bird malaria parasites until priority was given to Marchiafava and Celli's genus name *Plasmodium.*

Labbé believed that the several species of bird malaria parasites described by various authors were really not distinct species and that one frequently assumed the characteristics of another. He thus suggested that the name *Proteosoma grassii* be used for all species previously described.

Following descriptions of the morphology and habitats of the blood parasites of vertebrates, Labbé directed his attention towards a discussion of the epidemiology, immunology and evolution of these organisms. He stated that experimental infection may be achieved by injecting parasitized blood from individual to individual, but not from species to species. We now know that this cannot be done with all of the blood parasites, particularly those in which only the gametocyte stage occurs in the peripheral blood, and we also know now that malaria parasites can be transferred from one species of bird to another.

Labbé's conception of immunity was not clear cut and he made no attempt to describe its mechanism. Of interest, however, is his statement that " phagocytosis, as a method of defense, is not generally in evidence; but in certain cases, the leucocytes acquire a phagocytic power." He therefore did not agree with the viewpoint expressed by Danilewsky with regard to the role of the white cells in malaria, namely that the phagocytes are of prime importance in combatting the disease.

Taken as a whole, the collection of facts and personal experiences given by Labbé in his monograph served to concentrate and orient the literature on intracorpuscular parasites from the time of Laveran's discovery to his own. His description of the parasites was similar to that which had been given by Danilewsky. Plate II is a photograph of Labbé's drawings of bird malaria parasites taken from his monograph (1894).

7. OPIE AND MacCALLUM

The endocorpuscular parasites of birds were not seriously considered in America until 13 years after their discovery in Europe. In 1898 Eugene Opie, of the Johns Hopkins University in Baltimore, published a paper on the haemocytozoa of birds. In this work a summary of the contemporary literature on bird malaria was presented, and a number of original observations were given.

Opie examined a number of species of native American birds and found malaria parasites in several of them. Of two great-horned owls (*Bubo virginianus*) examined, one was found to have a severe infection. Four out of five crows (*Corvus americanus*) were found to harbor similar organisms. Blackbirds and sparrows were found to be commonly infected. Double infections with the halter-shaped type of parasite (*Haemoproteus*) and Labbé's *Proteosoma* (*Plasmodium*) were observed in these birds.

Besides studying the incidence of malaria parasites in wild birds, Opie made careful observations on the morphology and physiology of the parasites. He described such familiar phenomena as the displacement of the host-cell nucleus by the parasite, the occurrence of more than one parasite within a single red cell, and the apparent forcing out of the nuclei of some host-cells by the parasites.

Immediately following Opie's work and appearing in the same volume of the Journal of Experimental Medicine were two papers by another Hopkins' investigator, W. G. MacCallum (1898). MacCallum mentioned the two types of organisms (*Halteridium* and *Proteosoma*) which occurred in the blood of birds, but in view of the preceding paper by Opie omitted detailed morphological descriptions of them. Of far more importance is his description of exflagellation and the union of gametes in the drawn blood of crows infected with *Haemoproteus*. This work had previously been reported in a short paper in 1897. Although *Haemoproteus* is not a true malaria parasite, this discovery was one of the most important in the history of malariology, since an analag-

ous process was soon observed in *Plasmodium falciparum* by MacCallum (1898), and a sexual cycle in malaria was thus demonstrated. In the same year (1898) the role of mosquitoes as vectors of malaria was described by Ross, a discovery based in part on MacCallum's description of the fertilization process. MacCallum also described the pathology of bird malaria infections, and gives a detailed account of changes in the internal organs due to the presence of malaria parasites. His account of the enlargement of the spleen and liver, pigmentation in these organs, and phagocytosis is one of the few detailed descriptions of bird malaria pathology which exist today.

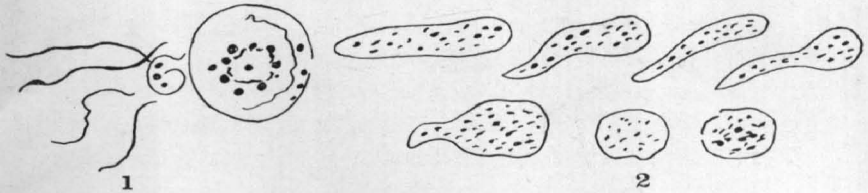
8. ROSS

The story of Sir Ronald Ross's brilliant researches in his attempts to find out how malaria was transmitted is one of the most interesting accounts in the history of science. The fact that mosquitoes might be involved in the transmission of malaria was suggested by several men before Ross actually demonstrated it. In 1848 Doctor Josiah Mott, of Mobile, Alabama, stated that yellow fever and malaria might be transmitted by mosquitoes, and six years later Beauperthuy concluded that both malaria and yellow fever (Venezuela) were produced by a venomus fluid injected under the skin by mosquitoes. Doctor A. F. A. King wrote a paper in 1883 in which he gives 19 reasons why mosquitoes probably carry malaria, and Laveran, about the same time, suggested that mosquitoes might play the same role in malaria as in filariasis. Ross's interest in the problem dated from his visit to Sir Patrick Manson in England in 1894, where he saw malaria parasites for the first time. Manson mentioned to Ross that he had formed the theory that mosquitoes carry malaria, and suggested that Ross attempt some experiments to demonstrate it. Ross first started to work on the mosquito problem in Secunderabad, India, with human malaria, and found that the crescents of malignant tertian malaria become " spherules " in the mosquito stomach. Flagellated

organisms were found in the mosquito, and oökinetes (" psorosperms ") and oöcysts (pigmented cells) were described. He saw male and female gametes united, but failed to recognize the significance of his observation in that he thought the male gamete was trying to get out of the cell (plate III, figure 1). After reading of MacCallum's work in 1897, he was further stimulated to continue his researches, but was transferred to a region in India where human malaria was not common. He therefore started experiments with sparrows and *Culex* mosquitoes, and completed the steps in the cycle which he had begun with the parasite of malignant tertian malaria in human beings. Sporozoites were found in oöcysts and these were traced to the salivary glands. This work was published in 1898. Were it not for the use of birds as experimental hosts it is doubtful whether the mosquito transmission of malaria would have been so early established. At any rate the discovery might have been delayed for a number of years, since Ross's experiments with mosquitoes and human malaria did not produce satisfactory results. It must also be noted that MacCallum's work had a direct bearing on Ross's discovery, since in going over MacCallum's description of exflagellation and fertilization in *Haemoproteus,* Ross was encouraged to continue his investigations, particularly with regard to the use of birds as experimental hosts. In plate III reproductions of some of Ross's original drawings are given, showing various stages in the sexual cycle of malaria.

9. FURTHER WORK BEFORE 1900

Several investigators other than those separately listed in the foregoing paragraphs were instrumental in advancing the knowledge of avian malariology before the beginning of the 20th Century. Sakharoff (1893) published his researches on the blood parasites of birds, and discussed the various views then held concerning the nature of flagellated bodies in freshly-drawn blood. Most of his work, however, was devoted to a study of *Leucocytozoon,* and therefore did not directly concern plasmodia. Di Mattei (1895) closely fol-



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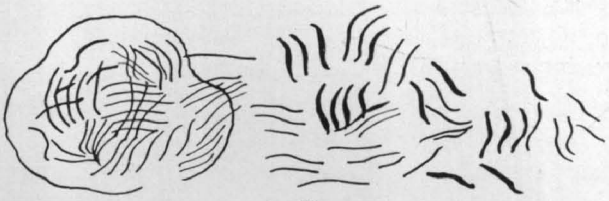

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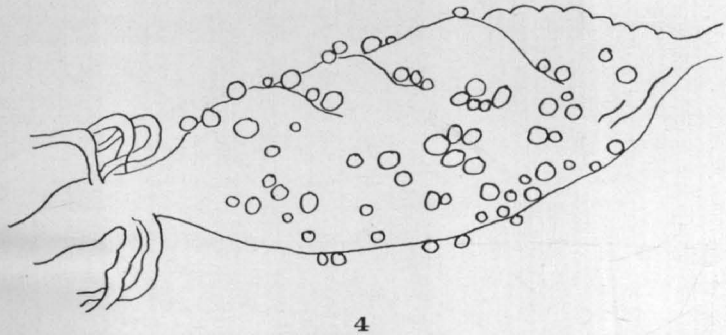

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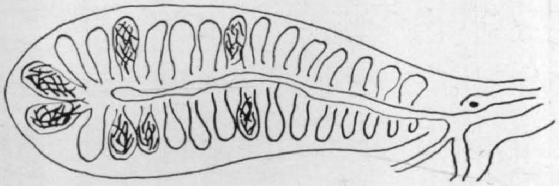

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PLATE III—Reproductions of several of Ross's original drawings showing various stages in the sexual cycle of malaria parasites, taken from his "Memoirs" (1923).

Figure 1. An exflagellating male gametocyte lying next to a female gamete (*P. malariae*). Ross did not know at the time he made this drawing (1897) that these were sex cells, and thought that the flagellated organism lying within the sphere, as illustrated, was trying to get out.

Figure 2. "Gregarines" from the stomach of mosquito pupae, and encysted "gregarines" containing "psorosperms" found at the end of the Malpighian tubules of pupae. Since these bodies were also found in adult mosquitoes, Ross believed at the time (1895) that they might have something to do with the cycle of malaria parasites.

Figure 3. "Germinal rods" (sporozoites) pouring out of a mature "coccidium" (oöcyst). Bird malaria (1898).

Figure 4. Stomach of a mosquito covered with oöcysts (bird malaria).

Figure 5. "Germinal rods" in the salivary gland of a mosquito (bird malaria, 1898).

lowed Grassi and Feletti's work with an extensive account of his studies on human and bird "malaria," although the pigeons which he used as laboratory hosts were infected with *Haemoproteus* and not *Plasmodium*. Studies were made on the temperature changes in normal and parasitized pigeons, and human malaria plasmodia were injected into pigeons with negative results. Ziemann (1898), Schaudinn (1898), and Koch (1899) published reviews of the literature on the blood parasites of man and birds. Koch verified the work of Ross, as did Daniels (1899), by successfully transmitting avian plasmodia from bird to bird by mosquitoes. It is of interest that Koch was the first worker to transmit bird malaria to canaries.

10. A BRIEF SUMMARY OF THE EARLY HISTORY OF BIRD MALARIA

1885.—Intracorpuscular parasites in the blood cells of birds were first described by Danilewsky. The name "pseudovacuoles" was given to the forms found in the interior of red blood cells. Exflagellation was described in birds, independently of Laveran's pre-existing work on human malaria.

1889.—Danilewsky's monograph "La Parasitologie Compareé du Sang" was published concerning observations made on the malaria parasites of wild birds obtained in Southern Russia.

1890.—Three more papers on bird malaria were published by Danilewsky in which phagocytosis and pathological lesions were described in detail. The difference between acute and chronic infections was also brought out.

Grassi and Feletti proposed the genus *Haemamoeba* for both human and bird malaria parasites.

Kruse proposed the generic name *Haemoproteus* for the crescent-shaped bodies found by Danilewsky in the blood cells of birds.

1891.—Laveran became interested in bird malaria and began to publish a series of papers on this subject, being

particularly interested in the use of birds as experimental laboratory hosts and the relationships between human and bird malaria parasites.

Celli and San Felice reclassified bird malaria parasites, but later work indicated that they were dealing with mixed infections of *Haemoproteus* and *Plasmodium*. They were the first to transfer infections from bird to bird by direct blood inoculation.

1894.—Labbé's monograph appeared in which blood-cell parasites were studied from a purely zoological point of view. He suggested the generic name *Proteosoma* for the parasites which Danilewsky described as *Cytosporon* and Grassi and Feletti as *Haemamoeba*.

1897.—MacCallum discovered the process of fertilization in *Haemoproteus*.

1898.—Opie reported the discovery of malaria parasites in North American birds. MacCallum described the pathology of bird malaria infections. Ross described the mosquito transmission of malaria through the use of birds as experimental hosts.

11. BIRD MALARIA STUDIES AFTER 1900

The preceding brief account of studies on bird malaria parasites before the beginning of the 20th century by no means includes all of the contributions which these early workers made to the knowledge of the subject. The results of their experiments will be presented in more detail under the separate divisions which are given in the pages to follow. The investigations which have been carried out from 1900 to the present time will be similarly treated. Several general trends in the problems studied and the methods used in bird malaria research occurred during these four decades, however, and in order to maintain the continuity of the historical background a brief outline of the development of research since 1900 will be presented briefly.

Ross's discovery of the mosquito transmission of malaria, and its confirmation by Daniels and Koch, should have

stimulated extensive experimentation with birds in order to elucidate some of the questions regarding the time relationships involved in the development of the malaria parasite in the mosquito, as well as bionomic and epidemiological studies on various species of mosquitoes. Surprisingly enough, however, this did not happen, and although a few short papers appeared which concerned the cycle of bird malaria in the invertebrate host most of the studies which immediately followed Ross's work were devoted to the course of malarial infections in birds and the immune responses of the vertebrate host, as well as to descriptions of new species and host records. If it were not for the frequent papers published by the Sergent brothers and their coworkers (beginning in 1904) from the Pasteur Institute in Algiers, the period from 1900 to 1923 would be remarkably barren in literature relative to bird malaria. It is true that other scientists showed interest in the avian plasmodia during this time, notably Ruge (1901), Wasielewski (1902, 1904), Novy and MacNeal (1904), Neumann (1908), Kopenharis (1911), Moldovan (1912), and Whitmore (1918, 1922), but there seems to have been no organized effort to transfer some of the problems encountered in human malaria to the experimental study of similar parasites which occurred in birds.

In 1923, however, Ben-Harel published the first paper on the nature of malarial infections in birds from the protozoology laboratories of the School of Hygiene and Public Health, the Johns Hopkins University, Baltimore, Md., and this was soon followed by a number of papers by other members of the same department, under the direction of Doctor Robert Hegner, as described in the introduction. These workers became associated with other universities in the United States, and some of them began research programs which resulted in several publications each year on various phases of bird malaria.

In Europe another stimulus resulted in an increased interest in bird malaria. The Great War had resulted in a shortage of quinine in Germany and chemists began to experi-

ment with synthetic derivatives showing the quinolene ring. An experimental animal was necessary for testing these compounds, and in 1926 Roehl announced his success with plasmochin on bird malaria. Immediately interest was aroused in the chemotherapy of bird malaria infections. All kinds of compounds were tried and in 1935 Kikuth found that an acridine dye, atebrin, would reduce the parasite number in malaria-infected birds.

In the meantime, the Italian school, including Missiroli, Raffaele, Corradetti, Giovannola, and others, were showing interest in bird malaria, and papers on various subjects appeared under their names. It is interesting to note that over three-fourths of all papers on bird malaria have appeared since 1923. During this time several stimuli have served to keep the interest in field alive, and the subject in its present state is still a fertile and stabile field for investigation.

CHAPTER II

GEOGRAPHICAL DISTRIBUTION, INCIDENCE AND HOST RECORDS

1. GEOGRAPHICAL DISTRIBUTION

Plasmodia in birds have been reported from many parts of the world, and wherever the blood of birds has been carefully searched malaria parasites have been found. In the early records of Danilewsky, Grassi and Feletti, Celli and San Felice, Opie, MacCallum, and others, it is difficult to determine whether all of the parasites found in different birds belonged to the genus *Plasmodium.* The first investigators in the field, as has already been pointed out, were not in agreement with regard to the classification of the parasites which they observed, and many of the first reports undoubtedly concerned *Haemoproteus* as well as *Plasmodium.* Subsequent workers, however, after learning how to distinguish these two genera, published host records which may be generally relied upon. Wild birds from England, Italy, Austria, Germany, France, Southern Russia, Switzerland, Greece, Algiers, Japan, Formosa, India, Ceylon, Africa, Mongolia, Australia, the Philippine Islands, Argentina, Brazil, Paraguay, Venezuela, Tonkin, Cuba, the Aru Isles, Java, Siberia, Mexico, and various parts of the United States have been found to harbor plasmodia. Many of these reports deal with but few blood examinations, but several extensive surveys have been made, especially in the United States. Representative examples are the work of Opie (1898) and MacCallum (1898) from Canada and Maryland; the Sergent brothers (1904a, 1905a) from Algiers; Novy and MacNeal (1904) from Ann Arbor, Michigan; Hegner and Chu (1930) and Russell (1932a) from the Philippine Islands; Cerny (1933) from Germany; Manwell and Herman (1935a and b) from Syracuse, New York; Herman (1938c) from Cape Cod; Coatney and Roudabush (1937), Coatney

and West (1938), and Coatney (1938c) from Nebraska; Huff (1939b) from birds obtained in various parts of the United States; Herms *et al* (1939) from California; and Beltrán (1940) and Hewitt (1940b) from Mexico.

TABLE 1

INCIDENCE OF PLASMODIA IN WILD BIRDS FROM VARIOUS PARTS OF THE WORLD

Authors	Locality	No. birds examined	No. species examined	No. birds positive for *Plasmodium*	Percentage of birds positive for *Plasmodium*
Opie and MacCallum (1898)	Maryland and Canada	125	6	16(?)	12.8
Sergents (1904)	Algiers	307	18	37	12.0
Sergents (1905)	Algiers	122	22	4	3.3
Ogawa (1912)	Japan	1478	54	15	1.0
Franchini (1924)	Italy	186	23	9	4.8
Schuurman and Huinink (1929)	Netherland Indies	448	14	11	2.2
Hegner and Chu (1930)	Philippines	95	47	3	3.1
Uegaki (1930)	Malaya	135	9	9	6.6
Schwetz (1931)	Belgian Congo	143	18	3	2.0
Russell (1932)	Philippines	604	46	60	10.0
Cerny (1933)	Germany	247	62	11	4.4
Manwell and Herman (1935)	Syracuse, N. Y.	652	34	54	8.2
Coatney and Roudabush (1937)	Nebraska	89	44	4	4.4
Coatney and West (1938)	Nebraska	84	35	4	4.7
Coatney (1938)	Nebraska	63	23	1	1.6
Herman (1938)	Cape Cod, Mass.	1485	23	54	3.7
Huff (1939)	Various parts of U. S.	324	32	59	18.2
Herms *et al.* (1939)	California	150	30	29	19.3
Beltrán (1940)	Mexico	85	7	7	8.2
Hewitt (1940)	Mexico	94	3	12	12.8
Totals.....		6916		402	5.8

Avian plasmodia undoubtedly occur in every part of the world where birds are to be found, except possibly in the far North. The climatic barriers which restrict the occurrence of human malaria parasites do not exist in bird malaria, since

the incidence of infection in birds is just as high in temperate zones as it is in tropical regions. The migratory habits of some birds probably increases the infection rate. Surveys in areas never before studied will probably reveal many new host records as well as species other than those now known.

2. INCIDENCE OF INFECTION IN WILD BIRDS

Data relative to the incidence of infection must necessarily be taken from reports involving a reasonably large series of birds. Furthermore, reports such as that of Cardamitis (1909), in which 936 wild birds were examined, cannot be considered, since a clear cut distinction is not made between *Plasmodium* and *Haemoproteus*. In table 1 data are presented from 20 surveys of blood protozoa in birds from different parts of the world. The variation which occurs with regard to the number of birds found positive for *Plasmodium* by the different investigators cited is not as great as might be expected, considering the numbers of birds examined in each survey and the methods used in detecting parasites. With the exceptions of papers by Manwell and Herman (1935b) and Hewitt (1940b), all of the data listed were obtained from peripheral blood smears only, yet the highest incidences are given by Herms *et al* (1939) and Huff (1939b), neither of whom examined the visceral organs. As a general rule, however, more positive cases are revealed when the viscera are examined or when subinoculations into parasite-free birds are made. Table 2 illustrates this point.

The incidence of infection in any series of birds examined also depends to a great extent upon the type of avian host. Plasmodia are known to occur more frequently in the passerine birds than in any other group. Sparrows have generally been found to be heavily infected in most regions where examinations have been made. Pigeons, doves, geese, ducks, turkeys, and chickens, however, seem to be rarely infected in nature, although malaria parasites are occasionally found in all of them. Beltrán (1939) recently examined 276 market birds (pigeons, chickens, and turkeys) in Mexico

City without finding a single malaria parasite. Herman (1938c) examined 85 common black ducks (*Anas rupripes tristis*), and 86 mourning doves (*Zenaidura macroura carolinensis*) in the Cape Cod region of Massachusetts, without finding malaria parasites. Huff (1939b) found only 2 in-

TABLE 2

SHOWING THE INCREASE IN POSITIVE DIAGNOSES FOR MALARIA IN WILD BIRDS WHEN SUBINOCULATIONS ARE MADE TO CANARIES, AS COMPARED WITH DIRECT BLOOD SMEARS (FROM MANWELL AND HERMAN, 1935).

Species of Bird	Number subinoculated from	Number showing positive smears	Infections in subinoculated birds		
			Pure	Mixed	Total
Bank swallow	12	..	..	..	..
Belted kingfisher	2	..	..	..	..
Bluebird	1	..	..	..	..
Catbird	1	1	1	..	1
Chimney swift	3	..	..	..	..
Cliff swallow	12	4	..	..	..
English sparrow	50	3	4	..	4
House wren	2	..	..	..	..
Purple grackle	5	1	..	..	..
Ring-necked pheasant	2	..	..	..	..
Robin	8	7	4	3	7
Slate-colored junco	11	1	1	1	2
Song sparrow	29	6	15	2	17
Starling	6	..	1	..	1
White-throated sparrow	2	2	1	1	2
Veery	1	..	..	..	..
Totals	147	25	27	7	34

fected mourning doves out of 190 examined. A strain of *P. relictum* has been isolated from pigeons by Coatney (1938b), and doves were also found to be infected with the same species of parasite.

Another factor which must be considered in interpreting differences in the incidence of malaria in birds is the presence or absence of suitable mosquito vectors. Several genera and species of mosquitoes are responsible for the transmission of

malaria in birds, but it is quite probable that suitable vectors are not equally distributed throughout all parts of the world.

Considering all of the above factors which help to explain the variations shown in table 1, the average incidence of 5.8 per cent positive for malaria parasites out of 6916 birds examined in different parts of the world represents a fair estimate of what might be expected from blood-smear examinations in almost any region where a survey is made.

Little is known about the effect of malaria on wild birds in nature; whether the parasites cause any great loss to the bird population is an open question. Manwell and Herman (1935c) make the following statement in this connection,

" It seems apparent that migratory birds are more commonly infected than those which do not migrate. Further study of the movements of infected birds through banding returns may shed much light on the nature of the infection and its effect on the seasonal activities of the bird, while, on the other hand, a greater knowledge of the parasites of banded birds will undoubtedly expand our present knowledge of the migratory and other habits of the host."

3. HOST RECORDS

The appended list of birds from which plasmodia have been described is taken chiefly from the extensive check lists given by Wenyon (1926), Coatney and Roudabush (1936), and Giovannola (1939). Several new host records have been added, to include reports which have appeared since the above lists were published; it is believed that the records are complete up to January 1, 1940.

Acomus erythrophthalmus: Scott, 1926.
Acrocephalus steperus: Nikitine and Artemenko, 1927.
Actophilus africanus: Hamerton, 1930.
Agelaius phoeniceus—red-winged blackbird: Opie, 1898; Herman, 1938.
Aidemosyne cantans: Scott, 1927,
Alauda arvensis—sky lark: Labbé, 1894; Doré, 1921; Nikitine and Artemenko, 1927.

Alauda cristata: Nikitine and Artemenko, 1927.
Aluco longimembris—grass owl: Russell, 1932.
Amadina erythrocephala: Scott, 1926.
Amadina fasciata—weaver finch: Hamerton, 1934.
Anthus novae-zealandiae—lark: Doré, 1920.
Antigone antigone—crane: Scott, 1926 (genus?).
Aphelocoma sordida—Swainson's blue jay: Plimmer, 1914
Aptenodytes patagonica: Scott, 1927.
Ara macao—red and blue macaw: Plimmer, 1912.
Aramides chiricota: Scott, 1926 (genus?).
Aramides cajanea cajanea: Lucena, 1939.
Ardeola grayi: Basu, 1938.
Argusianus argus: Scott, 1928; Hamerton, 1929.
Asio leucotis: Scott, 1926.
Astur badius: Hamerton, 1932.
Asturinula monogrammicus: Scott, 1927 (genus?).
Athene noctua—little owl: Sergents, 1906; Brumpt, 1909.
Atticora cyanolenca: Iturbe and González, 1916.
Balearica regulorum—crowned crane: Plimmer, 1912; Scott, 1926.
Barnardius semitorquata: Scott, 1927 (genus?).
Biziura lobata—musk duck: Gilruth, Sweet, and Dodd, 1910.
Bobo poensis: Scott, 1927.
Brachyspiza pileata: Lucena, 1938.
Calliste thoracica—yellow-breasted tanager: Plimmer, 1912.
Calospiza fastuosa—superb tanager: Scott, 1926; Hamerton, 1929.
Caprimulgus europaeus—night jar: Böing, 1925.
Cardinalis cardinalis: Schaudinn in Prowasek, 1911; Scott, 1927; Hamerton, 1932, 1933.
Carduelis carduelis—goldfinch: Koch, 1899.
Carduelis elegans—goldfinch: Plimmer, 1914; Nikitine and Artemenko, 1927.
Carduelis spinus: Cerny, 1933.
Carpodacus mexicanus—house finch: Plimmer, 1912; Herms *et al*, 1939; Beltrán, 1940; Hewitt, 1940.
Carpophaga concinna—blue-tailed fruit pigeon: Plimmer, 1912; Scott, 1926.
Cephalophoenus nasutus—large-nosed shrike: Hegner and Chu, 1930.
Cerchneis tinnunculus: Schaudinn in Prowasek, 1911.
Chamaepelia minuta: Scott, 1926 (genus?).
Chenopsis atrata—black swan: Cleland, 1915.
Chloris chloris: Franchini, 1924.

Chloropsis aurifrons: Scott, 1926; Hamerton, 1932.
Coccothraustes coccothraustes—hawfinch: Franchini, 1924; Böing, 1925.
Coccothraustes melanura—Japanese hawfinch: Plimmer, 1912.
Coccothraustes vulgaris: Nikitine and Artemenko, 1927.
Colaptes campestris: Carini and Maciel, 1916.
Columba palumbus—wood pigeon: Böing, 1925; Cerny, 1933.
Columbia grisea: Scott, 1927.
Columbia livia—pigeon: Sergents, 1904; Coatney, 1938.
Columbia sp.—pigeon: Carini, 1912.
Corvus b. brachyrhynchos—crow: Manwell and Herman, 1935; Coatney and West, 1938.
Corvus corax—raven: Plimmer, 1913.
Corvus frugilegus: Nikitine and Artemenko, 1927.
Coryllis pusillus: Hamerton, 1930.
Crateropus bicolor: Scott, 1927.
Cryptorhinus afra: Scott, 1927 (genus?).
Cursorius temmincki: Scott, 1927 (genus?).
Cyanops flavifrons—yellow-fronted barbel: Plimmer, 1924.
Cyanocitta cristata—blue jay: Coatney and Roudabush, 1937.
Cyanospiza leclancheri—rainbow bunting: Plimmer, 1912.
Cygnus melanocoryphus—swan: Schaudinn in Prowazek, 1911; Scott, 1928.
Dacnis cayana—blue sugar bird: Plimmer, 1912.
Dendroica pinus—pine warbler: Herman, 1938.
Dumatella carolinensis—catbird: Huff, 1935; Manwell, 1935; Manwell and Herman, 1935; Herman, 1938; Huff, 1939.
Dumetia hyperythra: Hamerton, 1930.
Emberiza citrinella—yellow hammer: Wasielewski, 1908; Cerny, 1933.
Emberiza fucata—bunting: Plimmer, 1913.
Emberiza projer: Wasielewski, 1902.
Emberiza variabilis—bunting: Ogawa, 1912.
Enneoctonus collurio: see *Lanius collurio*.
Erithragra musica: Scott, 1926.
Estrelda melpoda—orange-checked waxbill: Plimmer, 1912.
Estrilda coerulescens: Hamerton, 1931.
Estrilda cinerea: Scott, 1926.
Estrilda phoenicotis—waxbill: Marullaz, 1912; Scott, 1926.
Euethia candra: Scott, 1926.
Euethia canora: Hamerton, 1929.
Eulabes religiosa—starling: Scott, 1926; Hamerton, 1936.

Eunetta falcata—falcated duck: Plimmer, 1915.
Euphagus cyanocephalus—Brewer blackbird: Herms *et al,* 1939.
Euplocanus erythrophthalmus: Hamerton, 1929.
Excalfactoria lineata—island painted quail: Russell, 1932.
Falco hypolencus—grey falcon: Breinl, 1912.
Francolinus bicalcaratus: Scott, 1928.
Fringilla carduelis—goldfinch: Sergents, 1904.
Fringilla chloris: Wasielewski, 1902.
Fringilla coelebs—chaffinch: Wasielewski, 1902; Marullaz, 1912.
Fringilla kawarahiwa minor: Katahira, 1929.
Fringilla linota—linnet: Sergents, 1904.
Gallus domesticus—domestic fowl: Brumpt, 1935.
Gallus lafayettei: Hamerton, 1932.
Gallus varius: Scott, 1926 (genus?).
Garrulax leucophus—jay thrush: Plimmer. 1912.
Garrulus glandarius—common jay: Böing, 1925.
Gennaeus edwardsi—pheasant: Hamerton, 1930.
Geothlypis trichas brachidactyla—Maryland yellow throat: Huff, 1935; Huff, 1939.
Gracula religiosa—small hill mynah: Plimmer, 1912.
Haliastur indus: Hamerton, 1932.
Hirundo rustica: Nikitine and Artemenko, 1927.
Hylocichla musica—song-thrush: Coles, 1914.
Hylocichla mustelina—woodthrush: Wolfson, 1937.
Hypantorius cucullatus—weaver finch: Scott, 1926; Hamerton, 1930.
Icterus jamaicai—Brazilian hangnest: Plimmer, 1912.
Icterus tibialis: Hamerton, 1934.
Iole gularis—Philippine bulbul: Hegner and Chu, 1930.
Junco h. hyemalis—slate-colored junco: Manwell and Herman, 1935.
Lagonosticta senegala—weaver finch: Marullaz, 1912; Hamerton, 1929.
Lamprocolius australis: Scott, 1926.
Lamprotornis aenus (*L. caudatus*)—long-tailed glossy starling: Plimmer, 1912.
(*Lanius colluro*) *Enneoctonus collurio*—red-backed shrike: Böing, 1925.
Lanius excubitor—great grey shrike: Böing, 1925.
Ligurinus chloris—green finch: Ziemann, 1898.
Ligurinus crumeniferus: Scott, 1928.
Limnodromus griseus—dowitcher: Herman, 1938.
Liothrix luteus: Laveran and Marullaz, 1914; Scott, 1926; Hamerton, 1931.

Lophura i. igniti—fire-back pheasant: Coggeshall, 1938.
Lissotes maculipennis—bustard: Ross, 1911 (according to Coatney and Roudabush, 1936).
Loriculus indicus: Scott, 1926 (genus?).
Loxia curvirostra—crossbill: Plimmer, 1913.
Luscinia sp.—nightingale: Sergents, 1904.
Lyurus tetrix—blackgame: Böing, 1925.
Malimbus nitens: Scott, 1926 (genus?).
Megacephalon maleo: Hamerton, 1931.
Megaloprepia magnifica: Scott, 1927.
Meleagris sp.—turkey: Parcvanidze, 1914 (according to Coatney and Roudabush, 1936).
Melophus melanicterus—bunting: Plimmer, 1913.
Melophus melanocephalus: Scott, 1927.
Melospiza georgiana—song sparrow: Opie, 1898.
Melospiza m. melodia—song sparrow: Manwell and Herman, 1935; Herman, 1938; Huff, 1939.
Merula boulboul—grey-winged ouzel: Plimmer, 1915.
Mirafra javanica—Java lark: Schuurman and Huinink, 1929.
Molothrus ater—cowbird: Herman, 1938.
Molothrus sp.: Beltrán, 1940; Hewitt ,1940.
Motacilla alba: Nikitine and Artemenko, 1927.
Munia atricapilla: Scott, 1927.
Munia lineata—Calbani's weaver: Russell, 1932.
Munia maja: Scott, 1926; Uegaki, 1930.
Munia orizivora—sparrow: Mayer, 1910.
Munia punctulata: Hamerton, 1932.
Munia topela: Uegaki, 1930.
Myiarchus crinitus—crested flycatcher: Coatney and Roudabush, 1937.
Myristicivora bicolor: Scott, 1926 (genus?).
Neotis denhami: Scott, 1927.
Nothura maculosa: Scott, 1927.
Nucifraga caryocatactes—nutcracker: Plimmer, 1912.
Numenius variegatus—eastern whimbrel: Russell, 1932.
Numida sp.: Ross, 1911 (according to Coatney and Roudabush, 1936).
Numida ptilirhyncha—guinea fowl: Wenyon, 1908.
Nyctomassa violaceus: Scott, 1926 (genus?).
Ortygospiza polyzona—quail finch: Plimmer, 1915.
Oryzivora oryzivorae: Katahira, 1929.
Oryzornis oryzivora: Uegaki, 1930.

Otomela lucionensis—grey-headed shrike: Hegner and Chu, 1930.
Padda oryzivora—Java sparrow: Brumpt, 1935.
Paroaria cucullata—finch: Hamerton, 1932.
Parus major—great tit: Laveran, 1902.
Passer chloris—greenfinch: Sergents, 1904.
Passer domesticus—English sparrow: Grassi and Feletti, 1890, 1891; MacCallum, 1898; Opie, 1898; Johnston and Cleland, 1909; Grant, 1909; Cleland, 1915; Hartman, 1927; *et al.*
Passer hispaniolensis: Grassi and Feletti, 1890.
Passer montanus—tree sparrow: Wasielewski, 1902; Nikitine and Artemenko, 1927.
Passer sp.: Sergents, 1904; Mine, 1914.
Passerculus sandwichensis savanna—Savannah sparrow: Herman, 1938.
Passerina ciris—snowflake: Hamerton, 1932.
Perdix cinerea—partridge: Laveran and Lucet, 1905.
Perdix perdix: Böing, 1925.
Pernis apivorus—honey buzzard: Danilewsky, 1889.
Petrochelidon l. lunifrons—cliff swallow: Manwell, 1934.
Petrophila cinclorhyncha: Scott, 1927.
Phasianus colchicus—pheasant: Böing, 1925.
Phasianus mongolicus—Mongolian pheasant: Plimmer, 1912.
Phonipara canora—Cuban finch: Plimmer, 1912.
Pica pica—magpie: Coatney and Roudabush, 1937.
Pipilo erythrophthalmus—red-eyed towhee: Herman, 1938.
Pitta novae-guineae-pitta: Plimmer, 1917.
Planesticus anthracinus—thrush: Mazza and Fiora, 1930.
Plesiotagra culcullata: Hamerton, 1933.
Plocepasser mahali: Scott, 1926.
Ploceus baja: Uegaki, 1930.
Ploceus manjar—weaver bird: Schuurman and Huinink, 1929.
Pooecetes gramineus—vesper sparrow: Herman, 1938.
Pratincola caprata—chat: Plimmer, 1913.
Prinia extensicauda: Ogawa and Uegaki, 1927.
Progne subins—purple martin: Coatney and West, 1938.
Pryzivora crassirostris: Hamerton, 1929.
Psophia crepitans: Hamerton, 1932.
Ptelia melba: Hamerton, 1929.
Pternistes afer humboldti—francolin: Hamerton, 1929.
Pternistes nudicollis: Scott, 1927.
Ptilopus coronulatus: Scott, 1926 (genus?).
Pycnonotus jocosus—red-eared bulbul: Plimmer, 1912.

Pyrrhuloxia sinuata—Arizona pyrrhuloxia: Scott, 1928; Hamerton, 1933.
Pyrŏmelona franciscana: Marullaz, 1912; Hamerton, 1930.
Quele (Fondia?) aerythrops—fire bird: Marullaz, 1912.
Quiscalus quiscula—purple grackle: Manwell, 1934; Manwell and Herman, 1935; Herman, 1938; Huff, 1939.
Rallina eurizonoides—Philippine banded crake: Russell, 1932.
Rhamphocoelus braziliensis: Scott, 1926.
Rhea americana: Carini and Maciel, 1916.
Rhopodytes tristis: Scott, 1927.
Rollulus roulroul: Scott, 1928.
Saxicola aenanthe: Nikitine and Artemenko, 1927.
Saxicola salex saxilaris: Hoare, 1932.
Scardefela squamosa—scaly dove: Plimmer, 1902.
Scolopax rusticola: Scott, 1926.
Serina mozambicus: Hamerton, 1934.
Seriniculus molinus: Scott, 1926.
Serinus hortulanus: Scott, 1927.
Serinus icterus: Scott, 1927.
Seiurus n. noveboracensis—northern waterthrush: Herman, 1938.
Sialia sialis—bluebird: Scott, 1926, 1927; Huff, 1935; Huff, 1939.
Sitagra luteola—slender-billed weaver: Hamerton, 1933.
Spermospiza haematina: Hamerton, 1932.
Spheniscus demersus—penguin: Rodhain, 1937, 1938.
Spheniscus humbolti—penguin: Rodhain, 1937, 1938.
Spinus citrinellus—siskin: Schaudinn in Prowazek, 1911.
Spizella p. passerina—chipping sparrow: Manwell and Herman, 1935; Herman, 1938.
Spizella p. pusilla—field sparrow: Huff, 1939.
Sporaeginthus amandava—weaver finch: Hamerton, 1930.
Sporaeginthus melopodes: Scott, 1926.
Spreo superbus: Scott, 1926, 1927.
Sterna forsteri—Foster's tern: Coatney, 1938.
Stoparola melanops—fly-catcher: Plimmer, 1912.
Strix otus—long-eared owl: Wasielewski, 1902.
Strix flammea—barn owl: Sergents, 1906.
Sturnella magna—meadowlark: Huff, 1939.
Sturnus vulgaris—common starling: Manwell, 1934; Manwell and Herman, 1935.
Suthora gularis: Hamerton, 1931.
Sylvia atricapilla—blackcap warbler: Sergents, 1904.
Synallaxis ruficapilla: Carini and Maciel, 1916.

Syrmaticus reevesi: Scott, 1927.
Syrinum aluco—tawny owl: Sergents, 1906.
Syrnium nuchale—tawny owl: Sergents, 1906.
Textor alector—ox-bird: Plimmer, 1912.
Totanus eurhinus—Asiatic redshank: Russell, 1932.
Toxostoma cinereum—thrasher: Plimmer, 1913.
Tragopan satyra: Scott, 1926.
Trochalopterum taiwanum: Ogawa and Uegaki, 1927.
Troglodytes aedon—house-wren: Huff, 1939.
Turacus corythaix—white-crested touracou: Plimmer, 1914.
Turdus leucomelas: Lucena, 1938.
Turdus merula merula—blackbird: Giovannola, 1934.
Turdus migratorius—American robin: Novy and MacNeal, 1905; Plimmer, 1916; Manwell, 1934, 1935; Manwell and Herman, 1935; Huff, 1937; Coatney and Roudabush, 1937; Coatney and West, 1938; Herman, 1938; Huff, 1939.
Turdus musicus—song thrush: Doré, 1920.
Turdus mustelinus—wood thrush: Plimmer, 1914.
Turdus philomelos: Cerny, 1933.
Turdus pilaris: Kikuth, 1931.
Turdus rufiventris: Lucena, 1938.
Turnix fasciata—Philippine button quail: Russell, 1932.
Turtur auritus—dove: Sergents, 1904.
Turtur orientalis—dove: Ogawa, 1912.
Tympanistria tympanistria—dove: Scott, 1927; Hamerton, 1932.
Uraeginthus bengalus—weaver finch: Hamerton, 1934.
Zenaidura macroura macroura—mourning dove: Huff, 1935; Coatney, 1938; Huff, 1939.
Zonotrichia albicollis—white-throated sparrow: Manwell and Herman, Huff, 1939.
Zonotrichia coronata—golden-crowned sparrow: Huff, 1939.
Zonotrichia leucophrys gambelli: Scott, 1926; Herms *et al*, 1939.
Zonotrichia l. leucophrys—white-crowned sparrow: Huff, 1939.
Zosterops palpebrosa peguensis—white-eye: Ogawa and Uegaki, 1927.

CHAPTER III

EXPERIMENTAL HOSTS AND METHODS

A. EXPERIMENTAL HOSTS

Most of the early experimental work on bird malaria was done with sparrows, chaffinches, pigeons, linnets, and larks as laboratory hosts. Investigators could not be sure that they were dealing with parasite-free hosts, however, and the need for a more suitable bird became increasingly apparent. Koch (1899) appears to have been the first to investigate the possibilities of canaries and his contemporaries soon adopted the canary as the standard experimental host. During recent years Java sparrows, pigeons, ducks, and chickens have also become useful hosts for certain species of avian plasmodia, but the canary still retains the most important place in laboratories where species other than *Plasmodium gallinaceum* or *P. lophurae* are used for experimental purposes. For this reason it is considered desirable to present here data relative to the cost and maintenance of canaries as well as descriptions of the normal anatomy and physiology of this bird insofar as known.

1. COST AND MAINTENANCE OF CANARIES

The price of canaries differs considerably in various localities. Females are generally used, since they can be purchased at one-fourth the price of the males. For the past four years in Baltimore, Maryland, the Department of Protozoology of the School of Hygiene and Public Health has regularly paid from 50 cents to 75 cents apiece for female canaries. Out of many hundreds of birds which have been used none have been found to be naturally infected with malaria parasites. The custom of breeders to keep females in dark cellars or attics, usually well protected from the weather, generally guards them from mosquitoes which might carry infection.

It is generally desirable to contact a large pet supply house and place all orders through them, unless local breeders are unusually reliable.

The cost of feed runs from 8 cents to 12 cents per pound, and is most economically purchased in 100 pound lots if a large colony of birds is to be kept in stock. Cages and cage supplies may be purchased from the Arthur Hendryx Company, New Haven, Connecticut. An 11 in. x 7¼ in. x 10½ in. cage will hold from 1 to 6 canaries, and such a cage costs approximately 70 cents, including feed cups and perches.

2. DISEASES OF CANARIES

As a general rule the canary is a healthy bird under the usual laboratory conditions. With ordinary care and a constant supply of fresh food and water they are subject to few ills. Information concerning the care and treatment of diseased birds is presented in a paper by Alexander Wetmore (1924) of the U. S. Department of Agriculture (Farmer's Bulletin 1327, "Canaries, Their Care and Management").

3. NORMAL CANARY BLOOD AND TISSUES

Data relative to the anatomy and physiology of "normal" canaries is not abundant, and comparison standards for experimental work are badly needed. Some information has been published, however, and by comparing work which has been done on pigeons and chickens a fair normal standard has been obtained for investigations involving the pathology, hematology, cellular reactions, etc. of canaries infected with plasmodia.

a. *Body weight.* From measurements of 100 canaries Manwell (1930b) has found the average body weight to be about 16.5 grams. The range is between 12 and 18 grams.

b. *Erythrocyte counts.* From an average blood count of 23 canaries Young (1937a) reports 4,516,000 red cells per cubic millimeter. This agrees with the earlier report by Ben-Harel (1923) who found the normal erythrocyte count of

canaries to range from 4,500,000 to a little over 5,000,000 per cubic millimeter, the mean being somewhere around 4,500,000.

c. *Types of red blood cells.* Huff and Bloom (1935), Hegner and Hewitt (1937c, 1938), and Hegner and Eskridge (1938a) have described the types of red cells found in canary blood. Huff and Bloom (1935) describe: (1) basophil erythroblasts with deep blue-staining cytoplasm and large single or multiple nucleoli, (2) polychromatophil erythroblasts, slightly smaller than the first type with pale or blue-gray cytoplasm and progressively smaller nucleoli, (3) orthochromatic erythroblasts, showing an increase in orange-staining material in the cytoplasm, and with progressive reduction in nuclear size, and (4) mature, oval erythrocytes.

Hegner and Eskridge separate canary red cells into the following 5 types, according to their age when stained supravitally with brilliant cresyl blue followed by Giemsa's method:

(1). Type I. Spheroidal in shape, with a large nucleus in which the chromatin is well-scattered. The cytoplasm is basophilic and is characterized by the presence of a conspicuous reticulum. Only a fraction of 1 per cent of this type occurs in the blood of uninfected canaries.

(2). Type II. Ovoidal in shape, with the chromatin of the nucleus scattered in clumps and a polychromatophilic cytoplasm with a well defined reticulum. From 4 to 10 per cent of the red cells in normal canaries are of this type.

(3). Type III. Slightly smaller than type II, with a smaller and more pyknotic nucleus: The cytoplasm is generally orthochromatic, with less reticulum than type II cells. This type occurs in extremely variable numbers in normal canaries, but generally represents about 5 per cent of the total red-cell count.

(4). Type IV. Similar in shape and size to mature erythrocytes, with the exception that a small amount of reticulum remains in the cytoplasm.

(5). Type V. The mature erythrocyte, with nuclear chromatin closely packed into a homogeneous mass (pyknotic). The cytoplasm contains no reticulum.

Illustrations of these various red-cell types are given in plate I. Hegner and Hewitt (1937a) found that type II red cells become mature in the peripheral blood stream in approximately 24 hours.

d. *White blood cells.* Numerical or differential white-cell counts from normal canaries have apparently not been made. The morphology and types of leucocytes in canaries are similar to those of mammals with one outstanding exception; two kinds of eosinophiles occur, one with eosinophilic rods (pseudo- or special eosinophiles), and one with granules (true eosinophiles). Descriptions and colored illustrations of the white cell types in canaries are given by Huff and Bloom (1935).

e. *The spleen.* The spleen of normal canaries has been described in publications by the Sergents and Catanei (1929a), Cannon and W. H. Taliaferro (1931), Manwell (1932), Young (1938), Bloom and W. H. Taliaferro (1938), and Hewitt (1939d). A great deal of variation occurs in its macroscopic appearance. The Sergents and Catanei give the dimensions of the normal canary spleen as 2 mm. wide by 3 or 4 mm. long, and its average weight as 19 milligrams. Cannon and Taliaferro state that the average size is 2 mm. wide by 5 mm. long. In measurements of 38 normal spleens Hewitt found a variation of from 2 mm. to 5 mm. in width, and from 5 mm. to 15 mm. in length, the average being 3 mm. wide by 10 mm. long. Manwell found the mean weight of the spleen to be 36.2 milligrams. Young states that the spleen weighs from 19 to 25 milligrams (a ratio to total body weight of about 0.11 to 0.16 per cent). The average volume (as determined by displacement of Zenker's fluid) is given as 0.02 cc. by Young and 0.05 cc. by Hewitt. Its shape is usuallly cylindrical, and the color may vary from pale pink, almost white, through light pink to deep red. The capsule is glistening and transparent,

and through it can be seen blood vessels and follicles lying near the surface.

The histological structure of the canary spleen has never been carefully worked out, but there is reason to believe that it differs very little from the spleens of several of the small birds upon which detailed studies have been made. MacCallum (1898a), Nitsche (1929), Cannon and Taliaferro (1931), and Bloom and Taliaferro (1938) describe the essential differences between the avian spleen and mammalian spleen. A thin-walled capsule covers the entire organ except at the hilium where a thin strip of mesentery is attached. Trabeculae are rarely found. In the evolutionary scale it is in birds that splenic follicles first make their appearance, and in canaries they are sometimes quite prominent. The larger splenic vessels enter and leave the spleen at the hilium and branch laterally near the center of the organ, these branches ramifying to all parts of the pulp. Frequently small vessels lead to and from either end of the spleen, and these appear to supply the capsule. Sheaths of lymphoid tissue surround the arteries and can best be seen in sections where an artery has been cut transversely. The follicular arteries generally lie to one side of the follicles. The large central vein is the most conspicuous blood vessel and is usually well-filled with blood corpuscles. The structure of the red and white pulp is similar to that of mammals. The position of the spleen within the body of the canary is shown in plate IX.

f. *The liver.* Practically no information has been published regarding the size, weight and normal histology of the canary liver.

g. *The bone marrow.* The cancellous framework of the short and flat bones maintains its cellular character and is active in blood formation throughout the life of the canary In the long bones the cancellous framework is retained at the ends but a great deal of fat occurs in the center. Small islands of erythroblasts and myelocytes may be found in the cavities between the fat cells, and these are capable of multi-

plying very rapidly when special activity of the marrow is demanded. The following groups of cells may be distinguished in the marrow (Huff and Bloom, 1935): hemocytoblasts, the erythrocyte series, the granulocyte series, thrombocytes, fixed and free macrophages, monocytes, and plasma cells.

B. Experimental Methods

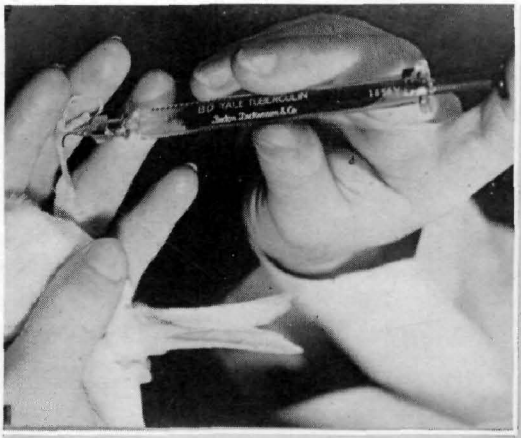
The mass of research material which has developed since Danilewsky first discovered malaria parasites in birds has introduced a set of distinctive experimental methods. The beginner in the field must acquaint himself with many procedures which are not described in ordinary texts on mammalian hematology, although the essential principles are fundamentally the same. The small size of the usual experimental host used introduces technical difficulties in experimental procedures which take considerable time to master, and the experienced avian malariologist must become familiar with a number of standard techniques peculiar to his field. Since the methods used by early investigators have been greatly improved upon, the following material represents only those techniques which have been found to be satisfactory by contemporary workers.

1. TRANSMISSION BY BLOOD INOCULATION

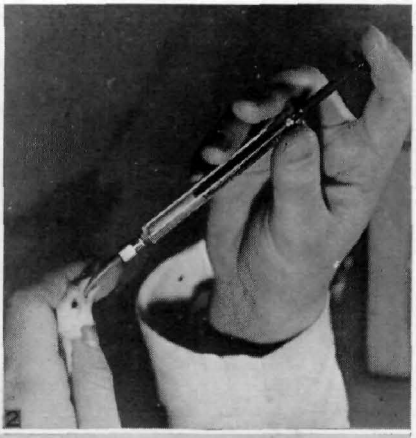
The transfer of parasites from an infected bird to a noninfected one can be made by mosquitoes or by direct blood inoculation. Complete procedures for rearing and infecting mosquitoes will be given in a later chapter. Direct blood transfer is usually employed for most experimental work, and may be achieved by either intraperitoneal, intramuscular, or intravenous inoculation. Each of these methods possesses usefulness, depending upon the type of infection desired.

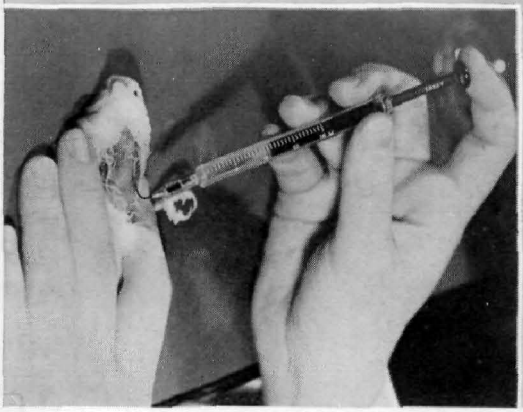
a. *Methods for obtaining infective blood.* When but one or two subinoculations are to be made a sufficient quantity of parasitized blood can be obtained from the leg vein or wing vein of an infected bird. To prevent the blood from

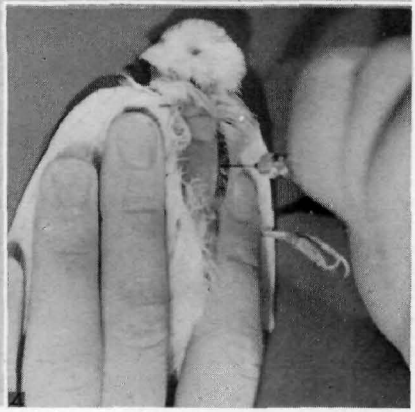
1.



2.







3.

4.

PLATE IV—Photographs demonstrating methods for: 1. Intraveneous inoculation with infected blood or drugs. 2. Oral injection with drugs. 3. Intramuscular inoculation with infected blood or drugs. 4. Heart puncture.

clotting, isotonic saline-citrate (0.85 per cent sodium chloride, 2.0 per cent sodium citrate), or a 0.001 per cent solution of heparin in 0.85 per cent saline may be used. A satisfactory proportion of saline-citrate to blood is 1 to 3 (1 part saline-citrate to 3 parts blood). A few drops of 0.001 per cent heparin will prevent coagulation.

The leg vein is pricked with a small Hagedorn needle and the blood is drawn into a 1 cc. Luer syringe fitted with a No. 27 hypodermic needle bent at right angles. Saline-citrate is introduced into the syringe before the blood is drawn. When a large number of birds are to be inoculated from a single donor it is convenient and time-saving to draw the required amount of blood directly from the heart. A straight No. 24 hypodermic needle may be used for this purpose; this is introduced into the body of the canary directly through the breast bone, halfway from either end of the keel of the sternum (plate IV, figure 4). From 600 to 800 milligrams of blood can be withdrawn in this manner, and since the total blood volume in canaries amounts to about 1000 milligrams the donor always dies. Larger amounts, up to 5 and 8 cc., may be drawn from the heart of a pigeon or a duck without apparent injury to the host.

b. *The sites and methods of inoculation.* Intra-peritoneal inoculation is rarely used, but is accomplished by inserting a straight No. 27 needle into the peritoneal cavity directly below the sternum. The breast muscle is the most convenient site for intramuscular inoculations and a bent No. 27 needle is used in this case (plate IV, figure 3). The feathers on the breast are first brushed back with 70 per cent alcohol.

The introduction of parasites directly into the blood stream requires considerably more practice than either of the other methods. The site generally chosen for inoculation is the tarso-metatarsal vein of the leg, between the foot and ankle joint (plate IV, figure 1). A bent No. 27 needle is introduced into the vein; it is advisable to rub the vein briskly for a few minutes before inoculation to increase its size. From 300 to 400 milligrams of blood and saline-citrate may be inoculated safely intravenously if care is taken, but doses

greater than this, or too rapid forcing of the plunger frequently cause complete collapse and death. Another intravenous site used by some workers is one of the small toe veins. A small piece of non-absorbent cotton is wrapped around the puncture to prevent excessive bleeding.

It is to be noted that careful precautions relative to asepsis are not necessary in any of the above procedures. The syringe and needle should be boiled if it has previously been filled with species of plasmodia different from that to be used a second time, and it is a good plan to boil syringes and needles before each inoculation regardless of the previous contents. Complete sterility of all instruments, however, is not necessary, since birds seem to be remarkably resistant to the usual types of bacterial infection.

Pigeons, ducks, chickens, etc. may all be inoculated according to one of the above methods.

2. THE NUMBER OF PARASITES NECESSARY TO PRODUCE INFECTIONS BY BLOOD INOCULATION

Edm. and Et. Sergent (1923) used varying doses of infective blood containing *P. relictum* in order to determine whether the size of the dose had any effect on the character of the resulting infections. The infective blood which they introduced into unparasitized birds contained parasite numbers varying from 1000 to 10,000, and although some of the infections produced varied in the height of the peak, they did not rise proportionately with the size of the dose. G. H. Boyd (1925), on the other hand, found a very definite correlation between the size of the dose of parasites given and the length of the prepatent period (table 6). A somewhat less pronounced correlation was observed between the number of parasites used for inoculation and the height of the peak of infection. Inoculations with small numbers of parasites (1,000 or less) produced infections only in a small number of cases.

Subsequent work by many investigators has demonstrated that when parasites can be readily found in the peripheral blood 30 milligrams inoculated intravenously into a parasite-

free canary will generally produce an infection. From 50 to 70 milligrams should be used for intramuscular or intraperitoneal inoculations.

For inoculations into larger birds (ducks, chickens, etc.) correspondingly larger doses of infective blood must be used, from 100 to 500 milligrams intravenously, depending upon (a) the size of the bird, and (b) the number of parasites present in the donor blood.

3. THE PREPARATION AND STAINING OF SLIDES

Blood films and tissue smears made from infected birds may be stained by one of several methods. Any of the Romanowsky blood stains are equally good; the success obtained depends to a great extent on the quality of the stain and the experience of the worker. Small drops of blood for staining are easily obtained from the leg, toe, or wing veins by pricking with a small Hagedorn needle.

a. *Giemsa's stain.* The stock solution is expensive to buy ready made, hence the following directions for its preparation are given:

Azur II-eosin (Grubler's or National Aniline)	3.0 gms.
Azur II (Grubler's or National Aniline)	0.8 gms.
Glycerin (Merck, c. p.)	200.0 gms.
Absolute methyl alcohol (c. p.)	250.0 gms.

Filter before use and store the filtrate in a tightly-stoppered bottle.

Blood films or tissue smears are fixed in absolute methyl alcohol for about 30 seconds, washed, and covered with diluted stain. Two or 3 drops of the stock solution are added to each cc. of water, and from 2 to 3 cc. of water are allowed for each slide to be stained. Buffered water with a pH of 6.8-7.0 gives the most dependable results. Stain for 30-45 minutes, wash the slides, and allow to dry in an upright position.

b. *Wright's stain.* This stain can be economically purchased ready-made, and its preparation in the laboratory is

time-consuming. Blood films are covered with the undiluted stock solution and after 1 minute (or as directed on the bottle) a quantity of buffered water (pH 6.8) equal to the amount of the stain used is added to each slide. After 3 or 4 minutes the stain is washed off and the slide is allowed to dry.

c. *MacNeal's tetrachrome stain.* Gingrich (1932) suggests the following modification of MacNeal's stain for blood films: prepare a stock solution by dissolving 1 gram of tetrachrome power in 50 cc. of c .p. glycerin, 50 cc. of c. p. methyl alcohol, and allow to stand for one or two days, then filter; fix the smear in absolute methyl alcohol (1 minute) and cover with diluted stain (3 drops of stock solution to 1 cc. of water) for 25 minutes or more, wash and dry the slide.

d. *Pappenheim's panoptic method.* Stain for one minute with May-Grunwald's (Jenner's) stain, add an equal quantity of water, and stain for 15 minutes with diluted Giemsa's stain. Wright's stain diluted with an equal quantity of water may be substituted for the Giemsa solution (stain only 5 minutes if Wright's is used).

4. SPECIAL STAINING METHODS

a. *Wet films.* Moist smears of blood and tissues may be fixed in Zenker-formol fluid, and stained with Delafield's hematoxylin followed by eosin-azur II (Huff and Bloom, 1935) or Heidenhain's iron haematoxylin without counterstaining with eosin-azur II. Neutral damar in xylol is recommended for mounting.

The following procedure has also given satisfactory results in this laboratory for staining wet films (Dr. T. T. Chen):

(1). Fix wet films in osmic acid vapor (2% solution) for 20 minutes.

(2). Stain with Giemsa's stain for 40 minutes (2% solution of stock in buffered water—pH 6.8).

(3). 100% dioxan—5 minutes.
(4). 100% dioxan—2nd change—10 minutes.
(5). Xylol—10 minutes.
(6). Mount in neutral balsam.

b. *Supravital staining.* A saturated solution of brilliant cresyl blue (National Aniline "vital stain") in 0.85 per cent saline and 2.0 per cent sodium citrate is used to demonstrate reticulum in immature avian red cells. Three methods are recommended by Hegner and Eskridge (1938a) for preparing permanent slides:

(1). A drop of brilliant cresyl blue is placed near the end of a slide; then a drop of blood is obtained by pricking the leg vein of the bird; this is taken up on the end of a toothpick and stirred into the drop of dye; finally, after about 10 seconds, a smear is made. The smear is dried, and stained by the usual Giemsa method.

(2). A second method of staining the red cells with brilliant cresyl blue is more effective but requires both more blood and more time. However, to determine the exact amount of reticulum present this method is very constant. Two hundred milligrams of brilliant cresyl blue solution are drawn into a 1 cc. syringe to which a needle has not been attached; from one to three drops of blood are then sucked into the syringe from the leg vein of a bird; the mixture is placed in a small, round-bottomed vial, allowed to settle for about 15 minutes and then centrifuged slowly for 5 minutes; the supernatant fluid is drawn off with a clean pipette leaving equal parts of blood cells and stain; finally this is thoroughly mixed, a drop is placed on a slide, and a smear is made and stained with Giemsa's stain.

(3). The last method requires the same amount of blood as in an ordinary smear and little extra time. One small drop of brilliant cresyl blue is drawn into a tuberculin syringe without a needle. The leg vein of the canary is pricked and a small drop of blood is drawn into the syringe. The blood and stain may remain in the syringe for from 1 minute to an

hour, depending on the depth of the reticulum stain desired. The blood and stain are then expelled on a clean slide, smeared, and counterstained. This method has given uniformly good results.

Another procedure for preparing permanent supra-vital blood preparations from birds has recently been used with much success by various workers in this laboratory. Equal quantities of blood and saturated brilliant cresyl blue in saline-citrate are mixed on the end of a clean slide. This is then placed in a moist chamber for from 10 to 15 minutes before spreading the film, and is counterstained by the Giemsa method in the usual manner.

5. SECTIONING AND STAINING TISSUES

The preparation of stained tissue sections from birds infected with malaria parasites does not differ greatly from the descriptions which may be found in any text-book on histology. It is preferable to use Giemsa's stain, however, in order to properly differentiate the parasites from the host cells; the following procedure is suggested by Hewitt (1939b):

a. *Source of material.* Upon autopsy small pieces of liver and entire spleens (from canaries) are dropped into the fixing fluid. Bone marrow is obtained by removing one of the long bones, cutting both ends, cleaning off the muscle, inserting a needle into one end of the bone, and slowly forcing the marrow out of the other end.

b. *Fixation and dehydration.* Zenker's fluid containing 5 per cent formol may be used as the fixing fluid. Tissues are allowed to fix for from 18 to 24 hours. After washing and removing the bichloride of mercury with iodinized alcohol, the usual method for dehydrating in alcohols and embedding in paraffin is followed.

c. *Sectioning and staining.* Sections are cut from 5 micra to 10 micra thick. In the thinner sections much more detail can be expected in the finished preparation, but 10 micra

sections are satisfactory for routine work. Sections are passed through xylol, absolute alcohol, 95 per cent alcohol, and 70 per cent alcohol, into distilled water. They are then mordanted in a 2.5 per cent solution of potassium bichromate for from ½ to 1 hour. Following a quick wash in distilled water they are immersed in the following stain:

Distilled water	100 cc.
0.5 per cent Na_2CO_3	2-4 drops.
Methyl alcohol (c p.)	3 cc.
Giemsa's stain	2.5 cc.

The stock solution of Giemsa's stain is prepared as earlier described. Sections are left in the stain for 24 hours.

d. *Differentiation.* Seventy per cent alcohol is used for differentiating after the excess stain has been removed from the slides by washing in distilled water colored lemon yellow with 2.5 per cent potassium bichromate. The differentiating process is the most critical step in the entire procedure and varies with different types of tissue. For example, sections of normal canary spleen require longer destaining than do sections of enlarged hemorrhagic spleens from acute infections. Furthermore, thin sections require less destaining than do thick sections. Liver sections are likewise quickly destained. It is therefore evident that this step is dependent upon the individual, but only a few trials are necessary before the process can be perfected. In general it may be said that as soon as the stain is being removed in noticable quantities by the alcohol (usually requiring from 30 seconds to 2 minutes) the slide can be washed quickly in distilled water, passed through the dehydrating mixtures and mounted. Tissues which contain a great deal of blood show sharply differentiated red and blue areas macroscopically when they are properly differentiated.

e. *Dehydrating and mounting.* Alcohols can not be used for dehydrating tissues stained with Giemsa's stain, because too much of the dye is removed in passing through the different concentrations. Xylol-acetone mixtures, however, are

satisfactory. The following dehydration and clearing procedure is recommended:

Distilled water (after differentiating)	wash
5% xylol, 95% acetone	1 minute
30% xylol, 70% acetone	2 minutes
70% xylol, 30% acetone	2 minutes
Xylol	5 minutes

Fresh mixtures containing 5 per cent xylol and 95 per cent acetone must be used since this fluid is apt to destain tissues slightly if used over and over again.

Neutral balsam or cedar oil is used for mounting.

6. ADMINISTRATION OF DRUGS AND CHEMICALS

Drugs and other chemicals are given to birds orally, intravenously, or intramuscularly. For the last two methods the same technique described under methods for transferring infections is used. Oral injections, as described by G. H. Boyd (1926) and Roehl (1926) are administered by attaching a short piece of catheter tubing to the end of a hypodermic syringe and using this apparatus as an oesophageal tube (plate IV, figure 2). The rubber tubing may be thrust down the throat of the bird with little chance of injury if the head and neck are kept on a plane approximately parallel to the body. Care must be taken that the tube does not enter the trachea since suffocation will result. The dosage of various drugs and chemicals will be given in a later section.

7. CULTIVATION *IN VITRO*

Little work has been done to determine the conditions necessary for growth *in vitro* of the bird malaria parasites. Bass and John's method, as devised for human malaria parasites, is not practical in the case of experimental infections in canaries, since these birds have so little blood. Manwell and Hewitt (1937) obtained retarded growth of *P. relictum* for at least one asexual generation in sealed capillary tubes, but were unable to demonstrate more than a single asexual cycle.

Hewitt (1938a), likewise, was able to secure segmenting stages of *P. cathemerium* from rings and young trophozoites on solid agar media covered with rabbit serum, but after the segmenters had reached maturity in their original host cells further development did not take place. The procedures thus far described are not suitable for routine laboratory use, and a practical method for *in vitro* cultivation is yet to be devised.

8. METHOD FOR COUNTING PARASITES

In attempts to express the degree of infection, several methods have been used by various investigators. Counts per cubic millimeter are not practical when working with small birds since the daily blood loss is too great. In chickens, ducks, and pigeons, however, this method is probably the best of any yet suggested.

A more common procedure suitable to small amounts of blood is that used by G. H. Boyd (1925), L. G. Taliaferro (1925), Gingrich (1932), Hartman (1927a) and others, namely, the expression of the parasite number in some chosen unit of blood cells. Counts are made of certain numbers of red blood cells and the parasites contained within them, and the number is usually then expressed in terms of 10,000 red cells. The blood sample taken will obviously need to be larger when few parasites are present than when most of the cells are parasitized. Gingrich (1932) computed that the probable error for observed values above 150 parasites per 10,000 red cells is 10 per cent or less; for those between 50 and 150 parasites per 10,000 red cells, 15 per cent or less; and 20 per cent or less for those below 50. The number of red cells to be counted in order for any observed parasite-erythrocyte ratio to have a probable error of 10 per cent is calculated by Hartman (1927a) and Gingrich (1932) according to the following formula: $N = 45.954 \frac{I-P}{P}$, in which N is the number of red cells to be counted, P is the number of parasites per sample, and I is the sample unit.

Many workers count from 500 to 1000 red blood cells and

their parasites throughout the infection, and express the number of parasites per 10,000 red cells. For estimates of the daily trend of parasite number curves this method is useful, even though not strictly accurate, but where the results of the experiment depends upon accurate number counts, an actual count of 2000 or 3000 red cells is indicated.

The method of counting parasites per given number of fields does not seem desirable, since the number of red cells varies greatly in different parts of the blood film.

9. THE USE OF BIRD MALARIA INFECTIONS IN THE CLASSROOM

The advantages of using bird malaria infections in the classroom for pointing out certain features of plasmodial infections which would otherwise be difficult to demonstrate has been discussed by Manwell (1934c). All parasitological laboratories are not equipped to show the characteristic features of human malaria infections, and infected birds offer an available substitute.

The writer has conducted protozoology and malariology laboratories in which species differences, blood pathology, visceral tissue pathology, rise and fall of parasite number, penetration of young red cells, study of the asexual cycle, and exflagellation of bird malaria parasites have all served as valuable supplements to the material given on human malaria.

CHAPTER IV

SPECIES OF BIRD MALARIA PARASITES

1. HISTORY

Malaria parasites in birds have been classified under approximately 40 different species names; it is the constant problem of workers in the field to sort out the "good" species from those which are synonyms or physiologically different strains of species already described. Danilewsky (1889) started out by calling the intracorpuscular parasites which he found in the blood of birds *pseudovacuoles.* The flagellated forms which he found in freshly-drawn blood were first named *pseudo-vermiculi* and later *Polymitus avium.* Grassi and Feletti (1890c, 1891) proposed the genus names *Haemamoeba* and *Laverania* for the two types of parasites which they found, and separated the latter genus into *L. danilewsky* in birds and *L. malariae* in man. *L. danilewsky* was later changed to *Halteridium danilewsky* by Labbé (1894), but it was found that Kruse (1890) had priority for the genus name of this type of parasite, namely, *Haemoproteus.* This completed one of the links in the nomenclatorial chain, but the genus *Haemamoeba* was not so readily disposed of. Grassi and Feletti (1890c) described *H. praecox* from man and birds, believing that it was the same species in both cases, but soon changed the name of the parasite in birds to *H. relicta* (1891c). Two other species from birds, *H. subpraecox* and *H. subimmaculata,* were described by the same writers. *H. relicta* segmented when it had reached its full size, and produced from 20 to 30 "gymnospores" (merozoites). *H. subpraecox* segmented before it completely filled the red cell, and from 5 to 12 merozoites were formed. The prefix was added to the species names of *H. subpraecox* and *H. subimmaculata* to distinguish the para-

sites from similar organisms found in human beings. Neither *H. immaculata* (man) or *H. subimmaculata* (birds) produced pigment. Celli and San Felice (1891) added to the confusion by describing three types of parasites observed in the blood cells of birds from what were probably mixed infections with *Plasmodium* and *Haemoproteus*. Fortunately, however, these authors did not suggest new generic or specific names.

A critical review of the literature then known was given by Labbé (1894). He sought to settle the species problem by putting the bird malaria parasites in the new genus *Proteosoma*, and gave them the specific name *grassii*. This nomenclature was accepted by many malariologists for years afterward, although the species name *relictum* was given preference to Labbé's proposed name *grassii*.

After it was generally recognized that Marchiafava and Celli's genus *Plasmodium* (1885) included the avian parasites as well as those in man, the problems of the taxonomist had just begun. Novy and MacNeal (1904) were the first to add another species to the list; they gave the name *Plasmodium vaughani* to a small parasite which they found in the blood cells of a robin. Their description of the parasite was very short; what is probably the same organism was more fully described by Laveran and Marullaz (1914) as *Haemamoeba tenuis* (*Plasmodium tenue*) from *Liothrix luteus*. Laveran (1902b) found another malaria parasite in the great tit (*Parus major*) which he called *Haemamoeba majoris* (*Plasmodium majoris*), but confused some of the stages with *Leucocytozoon*, which was also present. *P. wasielewskii* (Brumpt, 1909), *P. passeris* (Johnston and Cleland, 1909), *P. biziurae* (Gilruth, Sweet, and Dodd, 1910), and *P. columbae* (Carini, 1912) were soon added to the list, but none of these are now generally accepted as valid species.

Until Hartman's work in 1927 no further species were described, but this author recognized three avian plasmodia, *P. inconstans, P. praecox,* and *P. cathemerium*. An error in nomenclature here also resulted in considerable confusion.

PLATE V—Morphological characteristics used to identify avian plasmodia (x3500).

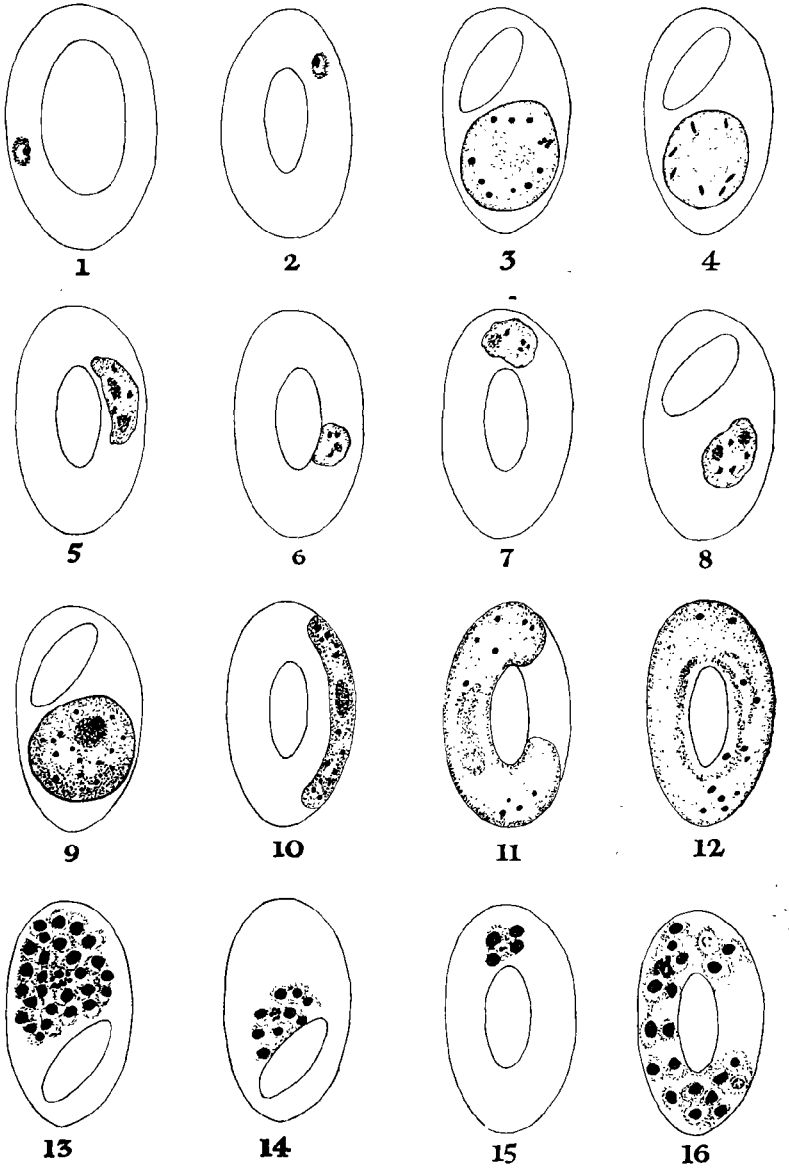
Figures 1 and 2. *Ring stages.* Figure 1 shows a ring stage in a young red cell. *P. cathemerium, P. relictum, P. elongatum, P. circumflexum,* and *P. nucleophilum* are the species known to penetrate young red cells. Figure 2 shows a ring stage in a mature red cell. *P. lophurae* seems to penetrate mature red cells, but nothing is known about other species in this respect.

Figures 3 and 4. *Differences in the character of the pigment.* Figure 3 is a male gametocyte of *P. relictum* and figure 4, *P. cathemerium.* Note that the pigment granules in *P. relictum* are round, and those of *P. cathemerium* are elongate. This is the chief morphological difference between these two species. The only other species with characteristic pigment is *P. vaughani,* which usually shows a bright, refractile grain of pigment, quite distinct from any other species.

Figures 5 to 8. *The shape and position of young trophozoites.* Figure 5 shows a small, elongate trophozoite which is characteristic of *P. circumflexum* and *P. lophurae.* Figure 6 is *P. nucleophilum,* closely applied to the nucleus of the host-cell. Figure 7 represents *P. polare,* which frequently occupies a polar position within the cell. The trophozoite in figure 8 has displaced the nucleus of the host-cell. This is characteristic of *P. relictum, P. cathemerium,* and *P. gallinaceum.*

Figures 9 to 12. *The shape of gametocytes.* Figure 9 shows a female gametocyte of *P. relictum.* The round shape and displacement of the host-nucleus is also characteristic of *P. gallinaceum* and *P. cathemerium.* The thin, elongate gametocyte of *P. elongatum,* shown in figure 10, is similar to that produced by *P. vaughani, P. oti, P. polare, P. rouxi,* and *P. hexamerium.* The large gametocytes in figures 11 and 12, which either partially or completely surround the host cell nucleus without generally displacing it, are characteristic of *P. circumflexum* and *P. lophurae.*

Figures 13 to 16. *Segmenters.* Figure 13 show the large segmenter of *P. gallinaceum.* As many as 30 merozoites occur in mature segmenters of this species. The host-cell nucleus is nearly always displaced. *P. relictum* and *P. cathemerium* are similar in this respect, but rarely produce more than 24 merozoites. Figure 14 shows a segmenter of *P. nucleophilum* closely applied to the nucleus of the host-cell. This is an example of a small segmenter which displaces the host-cell nucleus. Segmenters of *P. elongatum* may displace the red-cell nucleus but are generally not applied directly to it. Figure 15 illustrates the small segmenter of *P. rouxi* which characteristically produces only 4 merozoites. The segmenter shown in figure 16 is *P. circumflexum.* In both this species and in *P. lophurae* the host-cell nucleus may be partially or completely surrounded by the segmenting stage.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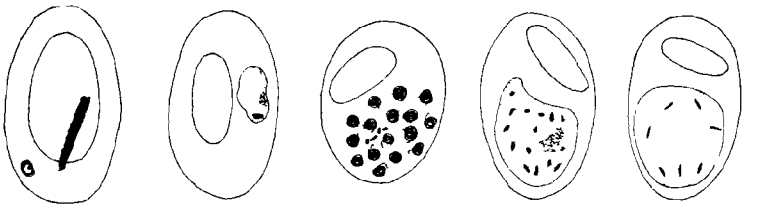
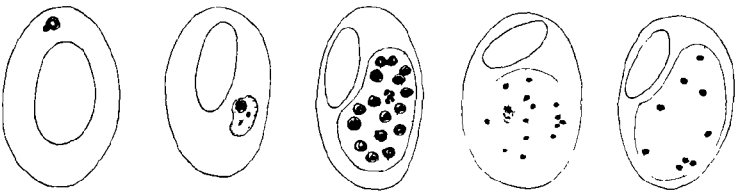
PLATES VI, VII, and VIII—Line drawings of the 12 species of bird malaria parasites which are recognized by most workers as "good" species. The first column on each plate represents ring stages or young trophozoites; the second column, older trophozoites or presegmenters; the third column, segmenters; the fourth column, female gametocytes; and the fifth column, male gametocytes (x2800). The sources are as follows:

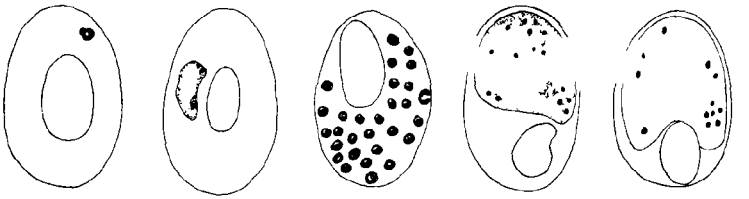
P. cathemerium, original; *P. relictum,* original; *P. gallinaceum,* after Giovannola (1938); *P. circumflexum,* original; *P. nucleophilum,* after Manwell and Voter (1939); *P. rouxi,* after Manwell (1935); *P. elongatum,* after Huff (1930); *P. lophurae,* after Coggeshall (1938); *P. oti,* after Wolfson (1936); *P. vaughani,* after Manwell (1935); *P. polare,* after Manwell (1935); and *P. hexamerium,* after Huff (1935).



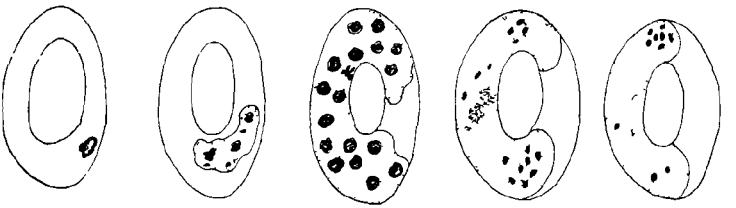
Plasmodium cathemerium



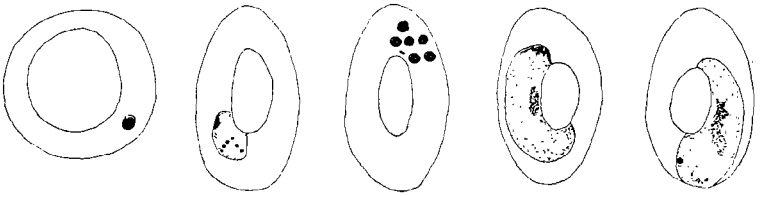
Plasmodium relictum



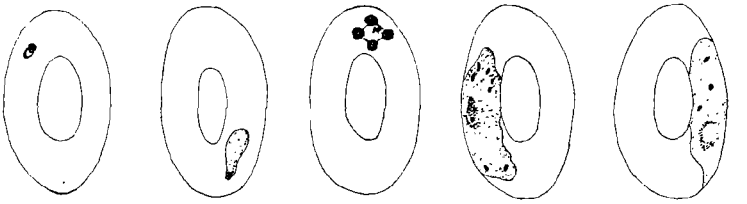
Plasmodium gallinaceum



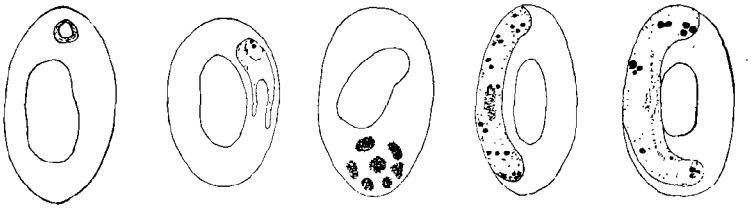
Plasmodium circumflexum



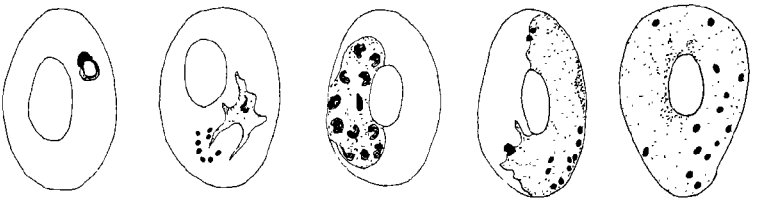
Plasmodium nucleophilum



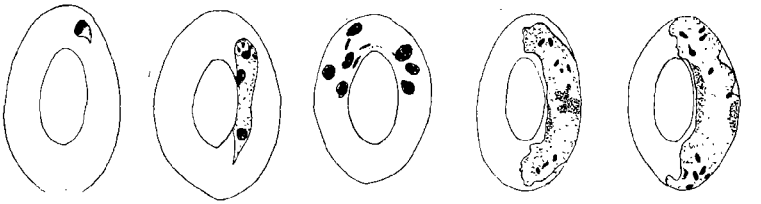
Plasmodium vouxi



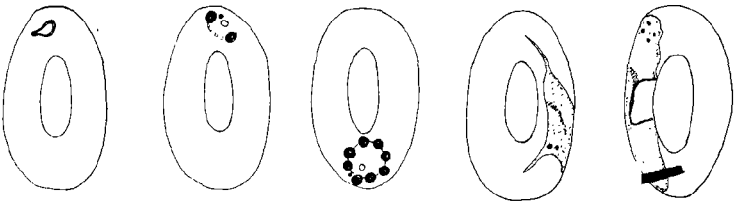
Plasmodium elongatum



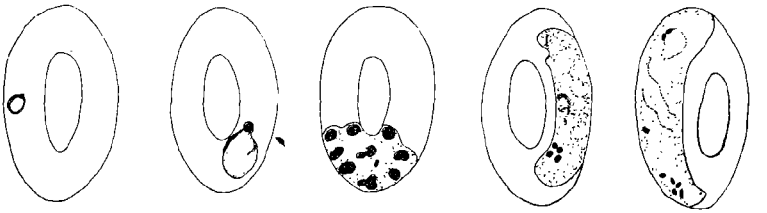
Plasmodium lophurae



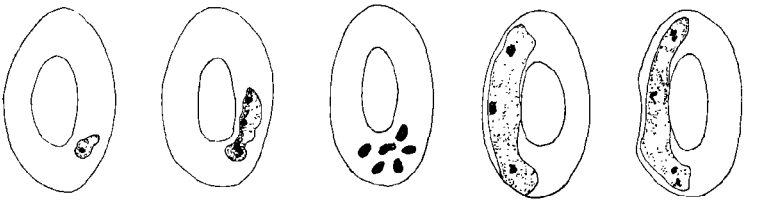
Plasmodium oti



Plasmodium vaughani



Plasmodium polare



Plasmodium hexamerium

Hartman (1927b) described two species with round gametocytes and one with elongate gametocytes. He named the first two species *P. inconstans* and *P. cathemerium,* but misinterpreted the name *Haemamoeba praecox,* which Grassi and Feletti first gave to what we now know as *P. falciparum* in man, a species with elongate gametocytes, and also to a similar parasite which they found in birds. The name given to the elongate parasite which Hartman described was therefore *P. praecox.* Huff (1930a) corrected this error, and called the elongate parasite *P. elongatum. P. cathemerium* was found to be a valid species, but *P. inconstans* is now considered to be a strain of *P. relictum.* In the meantime, the Sergent brothers and Catanei (1928) described *P. rouxi* from sparrows and canaries in Algiers.

In the past decade at least 10 new species have been added to the list of those already described, but some of these will undoubtedly be reduced to synonomy after more work is done with them. Little agreement exists among avian malariologists as to how many valid species actually have been found to date, and at least 7 papers have recently appeared which enumerate the distinguishing characteristics of avian plasmodia (Sergents and Catanei, 1931a; Manwell, 1935a, 1936a, 1938b; Giovannola, 1934b, 1939; and Brumpt, 1938a). Table 3 lists the species which have been described, together with the type hosts and type localities. Of the 31 species enumerated, 12 (in heavy type in the table) show characteristics which are constant enough in the opinion of most workers to separate them from other species. Plates VI, VII and VIII illustrate these 12 species. The remaining 19 species have either been reported only once, or have not yet been shown to be distinctly different from one of the valid species. Until more information is available concerning the appearance of these questionable forms and their life cycle in experimental hosts, they cannot be considered to be definitely established taxonomically.

TABLE 3

SPECIES OF AVIAN PLASMODIA

Species	Authors	Type Host	Type locality	Remarks
P. biziurae	Gilruth, Sweet, and Dodd (1910)	*Biziura lobata* (musk duck)	Australia	+
P. capistrani	Russell (1932)	*Excalfactoria lineata* (Island painted quail)	Philippines	syn. P. R.
P. CATHEMERIUM	Hartman (1927)	*Passer domesticus* (English sparrow)	Baltimore, Md.	
P. CIRCUMFLEXUM	Kikuth (1931)	*Turdus pilaris* (thrush)	Germany	
P. columbae	Carini (1912)	*Columbia* sp. (pigeon)	Brazil	+
P. ELONGATUM	Huff (1930)	*Passer domesticus* (English sparrow)	Baltimore, Md.	
P. fallax	Schwetz (1930)	*Syrinum nuchale* (owl)	Belgian Congo	++
P. GALLINACEUM	Brumpt (1935)	*Gallus domesticus* (common fowl)	Ceylon	
P. grassii	Labbé (1894)	*Alauda arvensis* (skylark)	France	syn. P. R.
P. heroni	Basu (1938)	*Ardeola grayi* (heron)	India	+
P. HEXAMERIUM	Huff (1935)	*Sialia s. sialis* (bluebird)	Kansas, Ill.	
P. subimmaculatus	Grassi and Feletti (1890)	*Falco timmusculus* (kestrel)	Sicily	+
P. inconstans	Hartman (1927)	*Passer domesticus* (English sparrow)	Virginia	syn. P. R.
P. LOPHURAE	Coggeshall (1938)	*Lophura i. igniti* (fire-back pheasant)	Borneo	
P. lutzi	Lucena (1939)	*Aramides c. cajanea*	Brazil	+
P. major	Raffaele (1930)	*Passer domesticus* (English sparrow)	Italy	syn. P. R.
P. majoris	Laveran (1902)	*Parus majoris* (Great Tit)	France	probably *Leucocytozoon*
P. malariae raupachi	Parcvanidze (1934)	*Meleagris* sp. (turkey)		+
P. NUCLEOPHILUM	Manwell (1935)	*Dumatella carolinensis* (catbird)	Syracuse, N. Y.	

Species	Authors	Type Host	Type locality	Remarks
P. OTI	Wolfson (1936)	*Asio otus naevius* (owl)	Baltimore, Md.	
P. paddae	Brumpt (1935)	*Padda oryzivora* (Java sparrow)	France	++
P. passeris	Johnson and Cleland (1909)	*Passer domesticus* (English sparrow)	Australia	syn. P. R.
P. POLARE	Manwell (1934)	*Petrochelidon l. lunifrons* (cliff swallow)	Westport, N. Y.	
P. praecox	Grassi and Feletti (1890)	*Passer domesticus* (English sparrow)	Italy	syn. P. R.
P. RELICTUM	Grassi and Feletti (1891)	*Passer domesticus* (English sparrow)	Italy	
P. ROUXI	Sergent, Edm. and Et., and Catanei (1928)	*Passer domesticus* (English sparrow)	Algiers	
P. subpraecox	Grassi and Feletti (1891)	*Passer domesticus* (English sparrow)	Italy	++
P. tenue	Laveran and Marullaz (1914)	*Liothrix luteus* (babbler)	Japan	syn. P. V.(?)
P. tumbayaensis	Mazza and Fiora (1930)	*Planesticus anthracinus* (thrush)	Argentina	syn. P. V.(?)
P. VAUGHANI	Novy and MacNeal (1908)	*Turdus migratorius* (robin)	Ann Arbor, Mich.	
P. wasielewskii	Brumpt (1909)	*Athene noctua* (owl)	France	++

Legend

syn. P. R. ..... synonym, *P. relictum*

syn. P. V. ..... synonym, *P. vaughani*

(+ ..... reported but a single time, and not confirmed by any other worker

++ ..... opinions differ greatly as to whether these are good species

Species in large type indicate those which are almost universally accepted.

2. CHARACTERISTICS USED TO IDENTIFY SPECIES

Every field of biology has its own criteria for the differentiation of species, and avian malariology is certainly not an exception in this respect. Morphological characteristics come first as a means of classification, but as pointed out by Manwell (1936a) the following factors must also be taken into account in classifying the plasmodia of birds: (1) host-parasite specificity; (2) the effect of the parasite on the erythrocyte; (3) the length of the asexual cycle; (4) pathogenicity; (5) reaction to anti-malarial drugs; (6) climatic conditions controlling development in the mosquito; (7) geographical distribution; and (8) types of cells parasitized. Cross-immunity reactions must also be added to this list. None of these characteristics are sufficient nomenclatorial criteria in themselves, but all of them, combined with the morphological appearance of the parasite, help to distinguish between different species (see plate V).

Of the morphological characteristics, size, the number and appearance of pigment granules, the number of merozoites produced per mature schizont, the shape of gametocytes, and staining differences are the most useful. Certain special characters, such as the refractile pigment granules in *P. vaughani* are also used. The shape of the gametocytes seems to be a constant and dependable character, and allows the separation of the species which have been described into two groups, those with round or oval gametocytes, and those with elongate gametocytes. This is of great help in diagnosing an unknown avian *Plasmodium,* since one entire group is eliminated at once. Manwell (1936a) suggests that the difference between these groups is so marked that two genera might well be recognized, *Plasmodium* (with round gametocytes), and *Laverania* (with elongate gametocytes). This, of course, was the original intention of Grassi and Feletti, in their genera *Haemamoeba* and *Laverania,* but they confused the issue by using the name *Laverania* for parasites which we now know to be *Haemoproteus.*

Following the separation into the round gametocyte or

oval gametocyte group, the next important step is to study the morphological characteristics of the asexual forms, as well as any of the physiological factors mentioned above which may be necessary. The following key, given by Manwell (1938b), will prove useful in the diagnosis of species. Table 8 is a

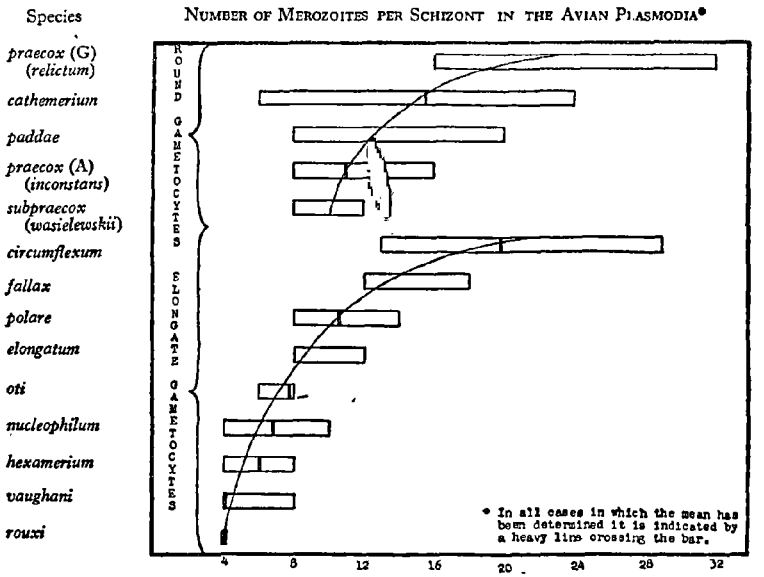


FIGURE 1—Number of merozoites per schizont in the avian plasmodia (from Manwell, 1936).

reproduction of a table published by Manwell (1938b), comparing infections produced in canaries by 10 different species of bird malaria. Several modifications and additions have been made in the key, but for the most part it is the same as that given by Manwell.

KEY TO SPECIES IDENTIFICATION

(Based chiefly on the morphology in the vertebrate host, as seen in stained blood smears.)

A. Gametocytes round or irregular 1

B. Gametocytes elongate 6

1, a. Both asexual and sexual stages tend to be round, displacing nucleus of host-cell 2

b. Schizonts tend to encircle the nucleus of the host-cell. 5

2, a. Canary not susceptible to infection 3

b. Canary susceptible to infection 4

3, a. Nucleus of host-cell displaced, but not very frequently expelled; pigment granules in gametocytes rather large and not very numerous; not known to occur in nature outside the Orient; 8-30 merozoites ..... *P. gallinaceum*

b. Nucleus of host-cell displaced and often expelled; pigment granules in gametocytes numerous and fine; 8-20 merozoites; so far known to occur only in the paddy bird ..... *P. paddae*

4, a. Pigment of gametocytes relatively fine and dot-like; nucleus of host-cell displaced, and often expelled, by larger forms; number of merozoites varies according to strain (reported to be 8-15 in one strain, 16-32 in another); very common in passerine birds ..... *P. relictum*

b. Pigment in gametocytes coarse and often elongate and rod-like nucleus of host-cell frequently expelled, particularly by mature gametocytes; merozoites vary from 6 to 24 per schizont; often marked quotidian periodicity; common among passerine birds, *P. cathemerium*

5, a. Nucleus of host-cell not displaced by asexual forms; 12-18 merozoites produced per schizont; reported so far only from the owl (*Syrnium nuchale*) of Africa ..... *P. fallax*

6, a. All stages common in peripheral blood; host-cell nucleus not displaced (except somewhat laterally by the larger gametocytes in certain cases) 7

b. Asexual stages not frequent in peripheral blood, occurring chiefly in the bone marrow; when found unusually seen in immature erythrocytes, but may parasitize any type of blood or blood-forming cells; host-cell nucleus not displaced by gametocytes, but asexual stages do so; segmenters (seen chiefly in the bone marrow) form 8-12 merozites..*P. elongatum*

7, a. Schizonts may produce more than four merozoites.. 8

b. Only four merozoites per schizont; parasite-level during chronic stage relatively high, so that parasites are usually easily found; not known to occur in nature except in Algerian sparrows; asexual forms contain generally two grains of pigment of unequal size; the smallest of the bird malaria parasites.*P. rouxi*

8. a. Generally more than four merozoites per schizont.. 9

b. Four to eight, usually four, merozoites per schizont; usually two unequal grains of pigment in asexual forms, of which the larger one often appears very sharply refractile; known to occur in nature only in the American robin and the starling (unless synonymous with *P. tenue*) ; very little larger than *P. rouxi* and very similar to it.....*P. vaughani*

9, a. Parasites not usually in contact with nucleus of host-cell 10

b. Larger stages, and particularly gametocytes, very frequently seen closely applied to the host-cell nucleus; mature schizonts rather rarely seen in the peripheral blood; not known to occur in any host other than the catbird; 4-9 merozoites per schizont (generally 6-8) .....*P. nucleophilum*

10, a, Schizonts usually produce 8 merozoites. Gametocytes and schizonts characteristically " ragged " in their outlines. Only known host—Eastern screech owl (*Otus asio naevius*).....*P. oti*

b. Schizonts usually produce more than 8 merozoites.. 11

c. Schizonts produce only 6 merozoites; larger trophozoites frequently elongated and assume oblique position across end of host-cell..... *P. hexamerium*

11, a. Schizonts usually produce more than 12 merozoites.. 12

b. Larger asexual forms characteristically at polar end of host-cell; schizonts often presenting a peculiar "stranded" appearance when nearly mature, and producing 8 or more merozoites when mature (average 10-11); host-cell nucleus not at all, or only very slightly displaced; parasites in peripheral blood usually very few in number in canary; as yet known to occur naturally only in cliff swallows, *P. polare*

12, a. Both sexual and asexual forms ten to encircle host-cell nucleus, but do not displace it; from 13-30 merozoites (average 19) per schizont; quite common in nature in a wide variety of hosts, *P. circumflexum*

b. Similar in general morphology to *P. circumflexum*, but usually with fewer merozoites per segmenter. Infective to chicks but not to canaries. Produces low-grade infections..... *P. lophurae*

3. STRAINS OF *P. RELICTUM* AND *P. CATHEMERIUM*

Since *P. relictum* and *P. cathemerium* are the species usually used for experimental work, and since they appear to occur more commonly than other species in nature, it is not surprising that a number of strains of each have been described. Some of these strains have been well defined and carried repeatedly through canaries, while others need considerably more study before a distinct differentiation can be made. In general it may be said that any malaria parasite which is isolated from a wild bird and subinoculated into an experimental laboratory host (canary, pigeon, duck, etc.) and is carried through the experimental host for a number of generations while its characteristics are studied deserves a strain designation. When a know strain is transferred from

one type of experimental host to a different type (e. g. canary to pigeon, or vice versa) with little change in its essential characteristics it does not seem necessary that a new strain designation be made.

Various strains of *P. relictum* and *P. cathemerium* are

TABLE 4

STRAINS OF *Plasmodium relictum*

Strain designation	Isolated by	Original host	Locality	Remarks
Type	Grassi and Feletti (1891)	sparrow	Sicily	Not carried through experimental animals.
A	Sergent brothers	?	Algiers	
B	Huff (1929)	sparrow	Boston, Mass.	
G	Institut für Schiffs- und Tropenkrankheiten	?	Hamburg, Germany	
R	Huff (1937)	robin	Kansas, Ill.	Segmentation matinal; short patent period; high percentage of gametocytes.
V	Huff (1926)	sparrow	Hampton, Va.	
W	Whitmore (1913)	sparrow	New York City	
Capistrani	Russell (1932)	quail	Philippines	
Inconstans	Huff (1927)	sparrow	Virginia	No clean-cut periodicity.
Mexican	Hewitt (1939)	finch	Mexico City	Similar to R.
Pigeon	Coatney (1938)	pigeon	Nebraska	Infective to pigeons, canaries, and fowl.
Woodthrush	Wolfson (1937)	woodthrush	Baltimore, Md.	Similar to R.

listed in tables 4 and 5, along with their source and differentiating characteristics, if the latter are clearly defined. Most of these strains are well known and have been used repeatedly for experimental material. In addition to the strains of *P. cathemerium* and *P. relictum* described several strains of *P. circumflexum* (Manwell and Goldstein, 1939c) are known.

TABLE 5

STRAINS OF *Plasmodium cathemerium*

Strain Designation	Isolated by	Original Host	Locality	Remarks
A	Huff (1933)	grackle	?	Greater virulence than type strain.
D	Hackett	?	Rome, Italy	From infected mosquitoes.
F	Huff and Gambrell (1934)	canary	Chicago, Ill.	Produces no gametocytes, variant of D strain.
H	Hartman (1924)	sparrow	Baltimore, Md.	Type strain.
M	Coatney and Roudabush (1937)	magpie	Nebraska	Similar to A.
N	Stauber	canary	Chicago, Ill.	Single-cell isolation.
Phenylhydrazine or Hartman-Hewitt	Hewitt (1939)	canary	Baltimore, Md.	Modified H strain, virulence seemingly increased through use of phenylhydrazine.

CHAPTER V

CHARACTERISTICS OF LABORATORY INFECTIONS

1. THE LIFE CYCLE

The cycle of avian plasmodia in the vertebrate and invertebrate hosts is comparable to that of the human malaria parasites. Briefly it is as follows: *sporozoites* present in the salivary glands of certain species of mosquitoes (*Culex, Aedes, Theobaldia* and *Anopheles*; see chapter IX) are injected into the blood of the bird while the insects are biting. The first parasite found in the red blood cells of the vertebrate host is in the " ring " stage (small *trophozoite*). This enlarges within the red cell and may eventually become either a *segmenter* or a *gametocyte*, the former representing the asexual division stage, and the latter the sexual stage, which undergoes further development in the insect host. The division products of each segmenter are known as *merozoites*. These escape from the red cell and penetrate new erythrocytes; the asexual cycle is then repeated. The haemoglobin within the red cells is apparently digested and by-products in the form of *pigment granules* are deposited within the parasites. The asexual cycle varies in length in the different species of plasmodia, as will be shown in a later section. A discussion will also be given later concerning the so-called " exoerythrocytic cycle " in bird malaria.

The gametocytes are of two types, male (*microgametocytes*) or female (*macrogametocytes*). When a mosquito ingests blood containing gametocytes, further development takes place. Each male gametocyte produces from 6 to 8 flagellated bodies (*male gametes*) in the stomach of the mosquito, and the female gametocyte undergoes a maturation process to become a *female gamete*. Fertilization is accomplished by the fusion of one male gamete with a female gamete, and the resulting *zygote* becomes an elongate *oökinete* capable of progressive movement. Oökinetes penetrate the stomach wall of the mosquito and come to rest just

beneath the outer epithelium, where they grow at the expense of the surrounding tissue. Within the resulting spherical *oöcysts* large numbers of minute, elongate cells called *sporozoites* develop, which eventually escape into the body cavity of the mosquito and may find their way to the salivary glands. Upon biting, the mosquito injects some of the sporozoites into the vertebrate host and the asexual cycle begins (see plate XIII). A further description of the cycle in the mosquito will be given in the section devoted to transmission and epidemiology.

The course of laboratory infections following direct blood transfer without intervening sexual stages in the mosquito may not be exactly comparable to the situation which occurs in nature. Most experimental infections are artificially induced, although some workers transmit parasites by means of infected mosquitoes. The latter procedure probably represents more closely the natural type of infection, but since the vast bulk of our knowledge has resulted from the former method, most of the data herein refers to infections produced by direct blood transfer. Differences recorded from mosquito-induced infections are noted when such material has been reported. Figures 3 and 4 illustrate different types of infections in canaries.

2. PARASITOLOGICAL PERIODS

The relations between host and parasite during infections with malaria parasites may be divided into two periods; one which refers to the reactions of the parasite in the host, the *parasitological period,* and the other descriptive of the reactions of the host to the parasite, the *clinical period.* The parasitological period is sub-divided into (a) the *prepatent period,* representing the time from the entrance of the parasite into the body until it can be demonstrated in the peripheral blood cells by ordinary diagnostic methods, (b) the *patent period,* which covers the interval during which parasites can be demonstrated in the blood, and (c) the *subpatent period,* when parasites cannot be recovered by the usual techniques employed, but may be present in very small numbers and can be demonstrated by special techniques (e. g.

subinoculation into unparasitized hosts). Secondary, tertiary, or more patent periods may follow the first subpatent period, and the time interval involved varies considerably. A diagrammatic representation of this is given in figure 2. Also shown in figure 2 are the clinical periods; (a) the *incubation period,* extending from the entrance of parasites into the host until symptoms appear, (b) the *period of symptoms,* (c) the *convalescent period,* and (d) the *period of relapse.* The last three are self-explanatory.

In birds infected with malaria the prepatent period is decidedly variable and depends to a great extent upon the technique employed in inoculation. Danilewsky, Grassi and Feletti, Labbé, etc. could not be sure about the length of the parasitological periods since they were dealing entirely with natural infections and had no way of knowing at what time their birds first acquired parasites. Danilewsky (1890c) stated that the severity of the infection has a direct relationship to the number of parasites present, but had no quantitative data to support his conclusion. In accord with Koch (1899) and Ruge (1901), he believed that bird malaria was of an acute nature and that after the disappearance of parasites from the peripheral blood, complete recovery resulted and immunity was established. Wasilewski (1904), Moldovan (1912), Whitmore (1918) and Prowazek (1920) believed that infections were chronic and could last for years, during which time any stage of the asexual cycle might be found in the peripheral blood stream.

Ben-Harel (1923) presented quantitative data relative to the course of infections. She performed her experiments with what we now believe to be a mixed infection with *P. relictum* and *P. elongatum* and encountered three types of infections; (a) primary acute infections, (b) extended irregular infections, and (c) benign infections. In the acute infections the prepatent period varied from 12 to 23 days after intramuscular or intraperitoneal inoculation with parasites, and the patent period varied from 5 to 10 days. Extended, irregular infections exhibited a prepatent period of from 12 to 14 days and the parasites remained present in the peripheral blood in demonstrable numbers for as long

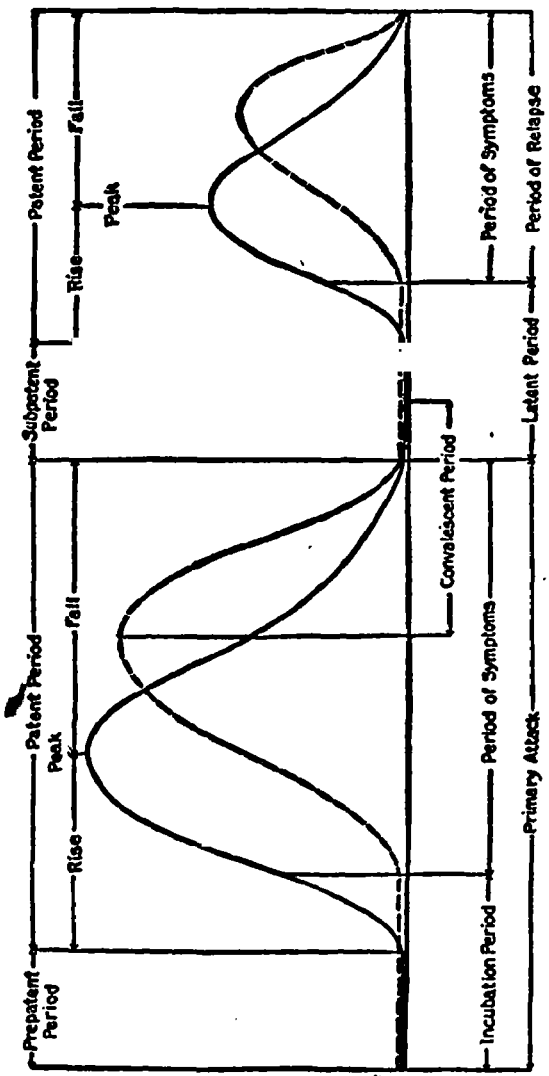


FIGURE 2—Diagrammatic representation of parasitological and clinical periods (from Hegner, Root, Augustine, and Huff, 1938).

as 23 days. In the benign infections the parasite number was low at all times; the prepatent period varied from 9 to 13 days, and the patent period continued for from 3 to 18

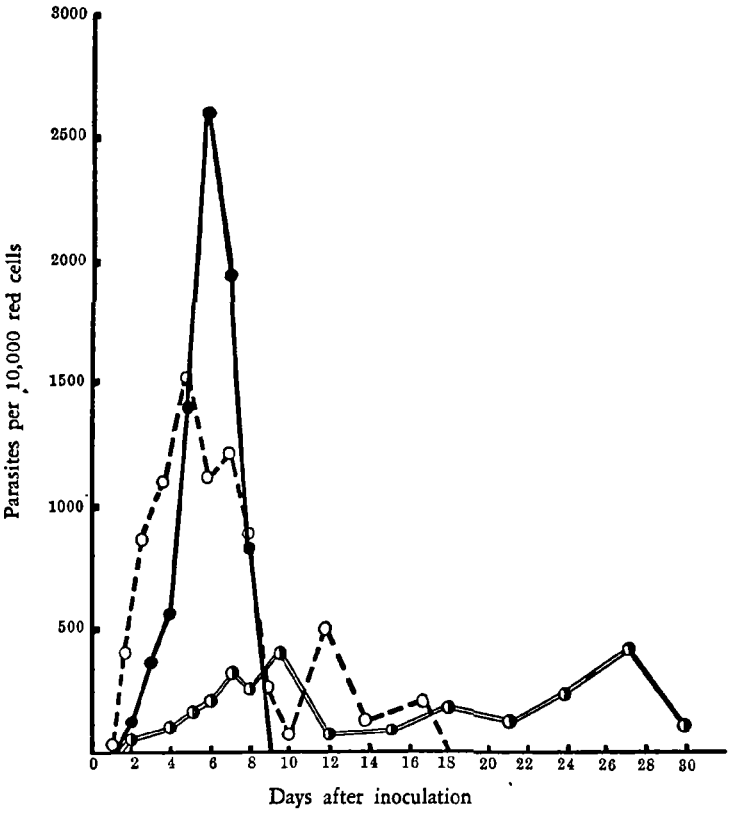


FIGURE 3—Types of infections produced by three different species of bird malaria parasites. The solid line represents the W strain of *P. relictum* (after Gingrich, 1933), the dotted line represents the H strain of *P. cathemerium*, and the double line is *P. rouxi* (after Gingrich, 1933).

days. The parasite number reached at the peak of the infections varied considerably, even though like doses of infective blood were inoculated. Both spontaneous and provocative relapses occurred in several birds; these will be discussed in the section on immunology.

L. G. Taliaferro's findings (1925) in infections with *P. relictum* confirmed Ben-Harel's description of the course of the disease in birds, although both the patent period and prepatent periods differed somewhat in each individual bird. G. H. Boyd (1925) succeeded in correlating the length of these periods and the height of infections with the number of parasites used for inoculation (table 6). Table 7 illustrates what he considers to be the average course of laboratory infections with *P. relictum.* The prepatent period is usually

TABLE 6

THE RELATION BETWEEN THE NUMBER OF PARASITES USED FOR INOCULATIONS AND THE LENGTH OF THE PREPATENT PERIOD (FROM G. H. BOYD, 1925)

Number of Parasites used for Inoculations	Length of the Prepatent Period in Days												
	1	2	3	4	5	6	7	8	9	10	11	12	
1,000	..	..	..	..	..	..	..	..	1	..	1	..	2
10,000	..	..	..	..	..	1	..	1	1	1	..	1	5
100,000	..	..	..	1	..	1	2	..	3	..	..	..	7
200,000	..	..	2	1	8	1	2	2	1	..	1	..	18
1,000,000	..	2	4	3	6	..	..	..	..	..	..	..	15
5,000,000	1	4	4	3	2	..	..	..	..	..	..	..	14
10,000,000	4	3	4	3	3	1	..	..	..	..	..	..	18
20,000,000	1	2	..	..	1	..	..	..	..	..	..	..	4
	6	11	14	11	20	4	4	3	6	1	2	1	83

about 5 days in infections with this species, the patent period about 10 days, and the average number of parasites present at the peak of infections is approximately 900 per 10,000 red blood cells. Figures 3 and 4 illustrate other types of infections.

Further work by many workers in infections with various species of bird malaria has shown that the course of infections can only be approximated, since individual variation is pronounced. Table 8, given by Manwell (1938b), compares the parasitological periods of 10 different species of bird

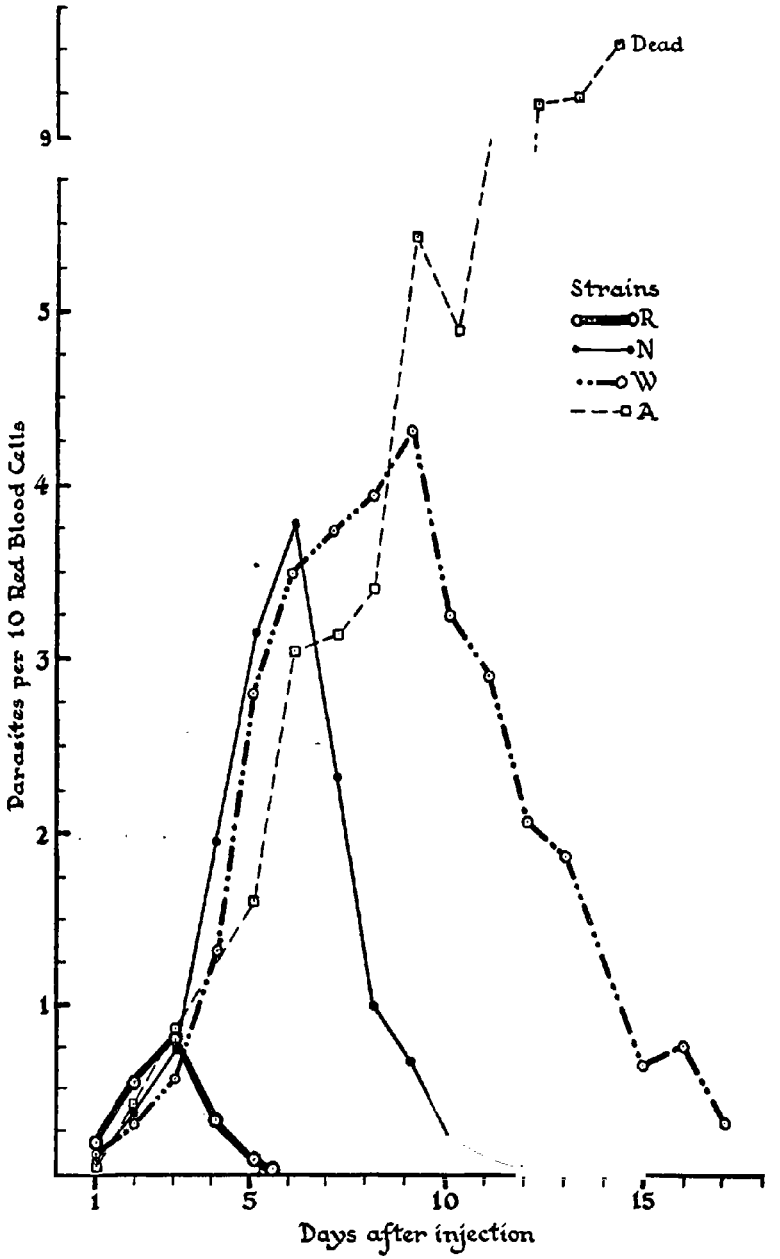


FIGURE 4—Comparison of normal infections of strains N and A of *P. cathemerium* and strains R and W of *P. relictum* (from Redmond, 1939).

TABLE 7

DATA FROM INFECTIONS WITH *P. relictum* TO ILLUSTRATE THE USUAL COURSE OF LABORATORY INFECTIONS WITH THIS SPECIES (FROM G. H. BOYD, 1925)

Infections	Prepatent period in days	Period of rise in days	Parasites per 10,000 red cells at peak	Length of crisis in days
103.....	4	4	125	10
104.....	3	5	330	8
118.....	1	6	1,650	7
123.....	4	4	1,430	5
130.....	7	5	1,110	4
147.....	2	6	660	5
150.....	3	5	200	5
151.....	3	5	1,000	5
154.....	6	4	100	3
155.....	3	7	2,500	5
163.....	2	4	670	6
166.....	4	5	3,330	Died at peak
180.....	3	5	400	3
190.....	5	10	5,000	Died at peak
194.....	9	6	400	5
196.....	11	2	10	1
206.....	3	5	125	3
212.....	8	10	500	12
213.....	5	6	150	11
217.....	9	5	2,000	4
218.....	9	8	250	11
222.....	6	4	10	2
226.....	11	7	3,330	Died at peak
234.....	4	5	50	2
237.....	5	6	75	2
238.....	5	2	10	2
276.....	5	7	100	5
277.....	4	5	1,330	Died at peak
281.....	8	3	15	2
282.....	8	4	50	2
Average.—	5.33	5.33	897	5

malaria, compiled from several authors. The factors which seem to govern the length of any particular parasitological period are (a) the number of viable parasites inoculated, (b) the site of inoculation, (c) the species of parasite, and (d) the degree of immunity evidenced by the host.

A discussion of the clinical periods will be taken up in another section.

3. TYPES OF RED CELLS PARASITIZED

After merozoites have escaped from mature segmenters it is necessary for them to penetrate new host cells in order to continue the asexual cycle. Danilewsky (1889, 1890c) noted that the immature red cells were more frequently parasitized than the mature cells, and that the growth of the parasite proceeded parallelly with the maturation of its host cell. Furthermore, he believed that since the young cells were attacked, the most obvious place for this to occur was in the bone marrow, spleen, and other hemopoeitic tissues, and confirmed his theory by finding parasites in the bone marrow when they could not be found in any other part of the body. This observation was not given serious consideration by subsequent workers, until 1923, when Ben-Harel again brought it to notice. She mentioned the phenomenon in the following words: " It was observed in this bird and in two previous birds with acute infections of a parallel nature, that only young red cells were parasitized. The old red cells which were in a small proportion were practically free from invasion." It might be mentioned that Hegner had previously made a similar observation (1918) but did not put it into print (Hegner and Hewitt, 1938). In 1925 Hegner referred to the situation in listing unsolved problems for research, but again the phenomenon did not attract immediate attention. Huff (1930a) mentioned the fact that the ring stages of *P. elongatum* were frequently located in erythroblasts, and this was soon confirmed by Raffaele (1934a) and Huff and Bloom (1935). These authors furthermore found that *P.*

elongatum is capable of living in all blood and blood-forming cells of the canary, including granular leucocytes.

Detailed studies of the relationship between young red cells and parasites, however, were first presented in papers by Hegner and Hewitt (1937, 1938), Hegner and Eskridge (1938a), and Hewitt (1938, 1939a and c). The red blood cells of canaries were classified into several types (see chapter III), and it was in the most immature types (polychromatophilic erythroblasts, types I and II) that ring stages were found directly after segmentation took place (plate I). As had previously been noted by Danilewsky, the parasites and red cells achieved maturity at approximately the same time (24 hours in the case of *P. cathemerium* infections). Several methods for demonstrating the penetration of immature red cells were devised, and four species of avian plasmodia (*P. cathemerium, P. relictum, P. circumflexum,* and *P. elongatum*) were found to behave similarly in that young red cells were penerated in each case. When phenylhydrazine hydrochloride (a blood-destroying chemical) was administered to birds before inoculation with parasites a great many young red cells were thrown into the peripheral blood. Infections in birds so treated were in general more severe than in control birds which did not receive the drug, presumably because more young red cells were available to parasitize (figure 5). Furthermore, the great susceptibility of young cells was demonstrated by the fact that many of them were infected with more than one parasite (figure 6). As many as 15 young trophozoites have been found within a single immature red cell. In phenylhydrazine-treated birds fewer multiple-infected cells occur due to the presence of large numbers of young cells (figure 7). This has an important bearing on the number of parasites which survive during the asexual cycle as will be shown later (figure 8 and table 9). Although several theoretical considerations have been offered to explain the reason for the penetration of young red cells by merozoites of several species of avian plasmodia none of these are as yet upheld by sufficient proof, and the reason for the phenomenon is not definitely known. Man-

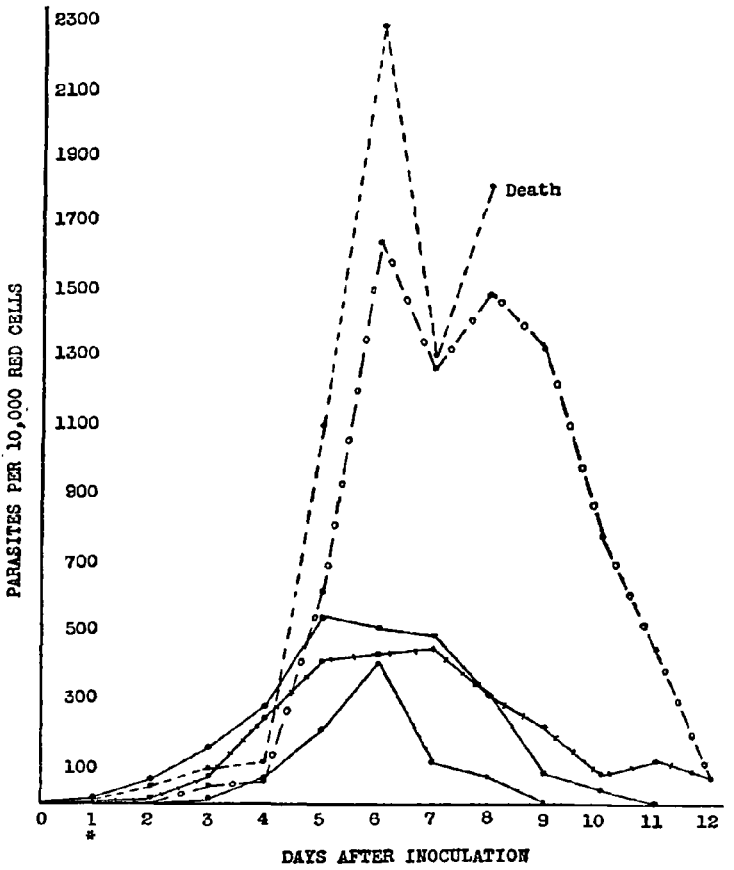


FIGURE 5—The effect of injections of phenylhydrazine hydrochloride on the severity of infections with *P. cathemerium*. Injections of phenylhydrazine are indicated by asterisks. The three lower curves represent control infections. (from Hegner and Hewitt, 1938).

well and Voter (1939) state that the ring stages of *P. nucleophilum* occur in young red cells more frequently than in old cells, but not to the same extent as in the above mentioned species.

Hegner (1938) gives a summary of the literature on human

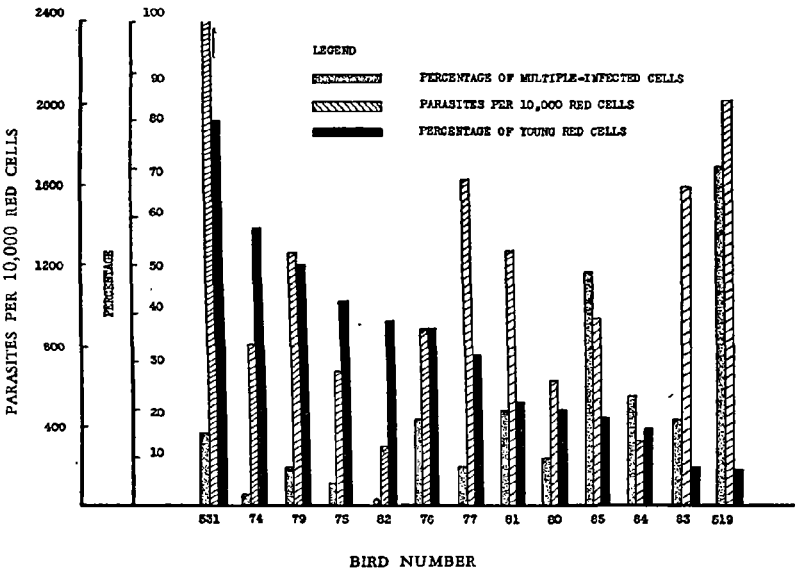


FIGURE 6—The relation between the percentage of young red cells, the number of parasites, and the percentage of multiple-infected red cells (from Hewitt, 1938).

malaria relative to this subject, and his own observations demonstrate that the ring stages of *P. vivax* are more frequently found in reticulocytes than in old red cells, whereas the rings of *P. falciparum* and *P. malariae* very often occur in mature erythrocytes. In a *P. knowlesi* infection in a monkey the rings occurred just as frequently in mature cells as in reticulocytes.

4. ASEXUAL REPRODUCTION AND PERIODICITY

a. *Definition of Terms.* The growth stages of bird malaria parasites within the red blood cells were recognized

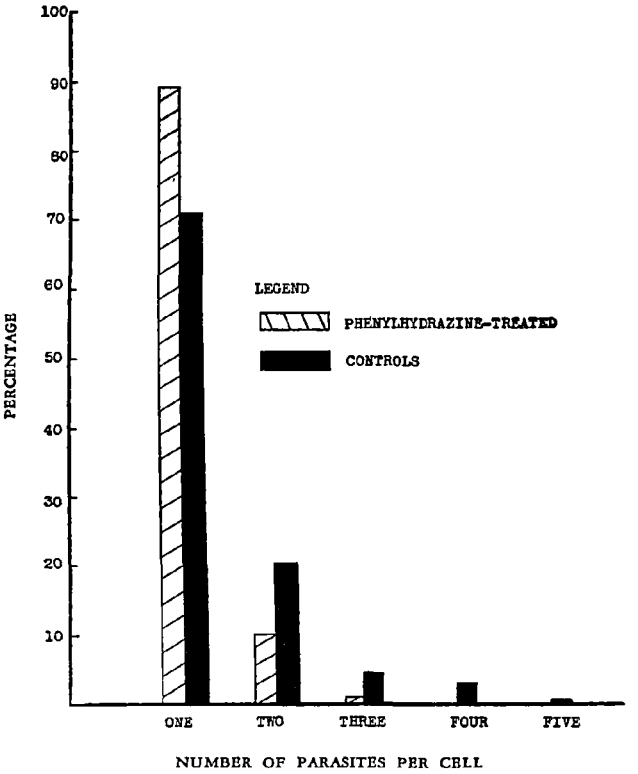


FIGURE 7—The percentage of multiple infections in six phenylhydrazine-treated birds and six controls on the 5th day of the patent period at 10 P. M. (from Hewitt, 1938).

by the pioneer workers in the field, but quantitative measurements of the time relationships involved in the maturation of ring stages to mature segmenters were not made until L. G. Taliaferro (1925) studied the periodicity and rate of repro-

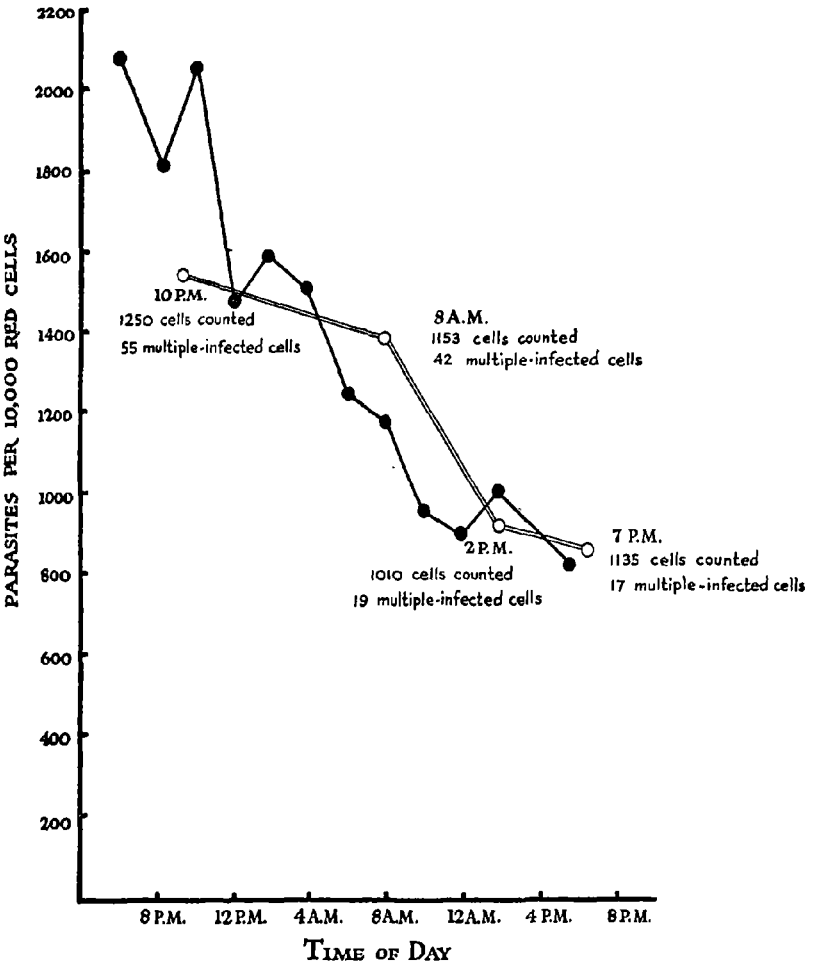


FIGURE 8—Hartman's curve (1927) showing the decline in parasite number during a single asexual cycle of *P. cathemerium* (solid line), and a curve showing the decrease in multiple-infected cells from a comparable infection with the same species (double line). Data regarding the number of multiple-infected cells is given at each point where counts were made (after Hegner and Hewitt, 1938).

duction of *P. cathemerium* in canaries. Drensky and Hegner (1926), Hartman (1927a), G. H. Boyd (1929, 1933), Gingrich (1929), Bovet (1930), G. H. Boyd and Allen (1934), Huff and Gambrell (1934), Lourie (1934c), Huff and Bloom (1935), Wolfson (1936a, 1937a), Giovannola (1938), Stauber (1939), and Coatney (1940) have confirmed Taliaferro's finding of regular periodic liberation of merozoites from mature segmenters in several species of avian plasmodia.

TABLE 9

DATA SHOWING THE DECREASE IN THE NUMBER OF MULTIPLE-INFECTED RED CELLS DURING THE ASEXUAL CYCLE (*P. cathemerium*)
(Compiled from data given by Hegner and Hewitt, 1938)

Time of day	Number of cells infected	Number of cells not infected	Number of Parasites per cell 6	5	4	3	2	1
10 P. M.[1]	76	1174	4 (5.3%)	2 (2.6%)	10 (13.2%)	20 (26.3%)	19 (25%)	21 (27.6%)
8 A. M.[2]	75	1049	0	7 (9.3%)	6 (8%)	9 (12%)	20 (26.2%)	33 (44%)
2 P. M.[3]	57	943	0	0	1 (1.8%)	6 (10.5%)	12 (21%)	38 (66.7%)
7 P. M.[4]	77	1056	0	0	0	1 (1.3%)	16 (20.8%)	60 (77.9%)

Legend: 1—ring stages, 2—young trophozoites, 3—presegmenters, 4—mature segmenters and gametocytes.

Wolfson (1936a) has conveniently defined *periodicity* as the degree of similarity in the length of the different asexual generations, and *synchronicity* as the degree of concurrence of any designated stage at any particular time. To illustrate these definitions, which are essential to a thorough understanding of the discussion of experimental results which follow, infections with *P. rouxi* in canaries may be cited. The Sergent brothers and Catanei (1929a) found that all growth stages of *P. rouxi* occur in the peripheral blood at all times. This is an example of low synchronicity, meaning that segmentation occurs continuously and at the same rate day and night. Wolfson (1936a), however, showed that there is

a definite correlation between the predominance of a particular growth stage and the hour of the day in *P. rouxi* infections, and that the degree of periodicity is marked, in that the period between the maximum prevalence of any particular growth stage is usually about 24 hours long. That all investigators do not recognize this distinction between synchronicity and periodicity is evidenced by statements in the literature which refer to a particular species as having no periodicity, when what is really meant is that no synchronicity was observed. All malaria parasites probably show regular periodicity, but greater diversities occur in the synchronicity of different strains and species.

b. *Methods for studying periodic phenomena.* Several methods have been used by different workers to ascertain the length of the asexual cycle. These may be outlined as follows:

(1) Evidence of equal distribution of parasites throughout the blood vessels of the host and their simultaneous segmentation.

(2). Methods based on changes in the mean area of parasites and the coefficient of variation.

(3). Methods based on changes in the number of mature segmenters.

(4). Methods based on changes in the number of asexual stages containing a given number of nuclei.

The first of these methods is purely qualitative and does not involve actual number counts. L. G. Taliaferro (1925) and Drensky and Hegner (1926) used the second method. Outlines of 100 parasites were drawn with the aid of the camera lucida at different times of the day and the mean size was then calculated for that particular observation. The product of the average length and average breadth was taken as the most accurate measure of size. These figures were then reproduced on graphs along with the coefficient of variation derived from the formula c. v. $= \frac{100\ \sigma}{M}$, in which σ represents the standard deviation and M the mean (figure 9).

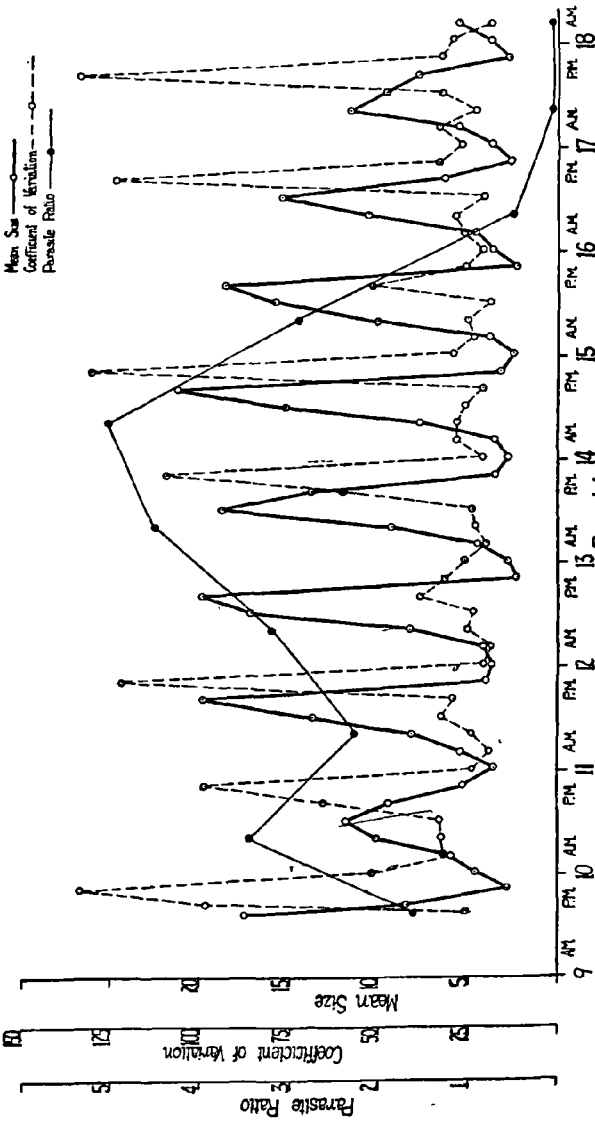


FIGURE 9—Mean size and coefficient of variation for asexual forms throughout an infection with *P. cathemerium* (from L. G. Taliaferro, 1925).

G. H. Boyd (1929, 1933), Lourie (1934c), Huff and Gambrell (1934), Wolfson (1936a, 1937a), Stauber (1939), and Coatney (1940) used the 3rd or 4th methods, simply by ascertaining the number of segmenting forms with a given number of nuclei at different times of the day and plotting the results on a graph. Sometimes only one stage was considered, but Wolfson (1936a, 1937a), particularly, used several growth stages to illustrate her results.

Any of these procedures produce satisfactory results in estimating the length of the asexual cycle. Observations should be made throughout the entire patent period, if possible, and on infections in a number of different birds.

c. *The length of the asexual cycle in different species.* Most of the work to date has been with *P. cathemerium*; this species completes a single asexual cycle in 24 hours. L. G. Taliaferro (1925) based her data on detailed studies of infections in 6 canaries, with additional corroborative evidence from 11 other infected birds. Figure 10 illustrates the growth stages of the strain of *P. cathemerium* which she used. The changes in mean size throughout the day show that the cycle of growth and development occurs regularly every 24 hours, and that segmentation takes place between 6 P. M. and 10 P. M. Drensky and Hegner (1926) confirmed these results, using the same strain of *P. cathemerium*; the work of Hartman, G. H. Boyd, G. H. Boyd and Allen, Huff and Gambrell, and Lourie further corroborates the 24-hour periodicity exhibited by this species. The degree of synchronicity in this original strain of *P. cathemerium* is very high, since the great majority of asexual parasites at any particular time are in the same stage of development.

The degree of synchronicity and length of the asexual cycle for 7 other species of avian plasmodia are given in table 10, compiled for the most part by Doctor Wolfson in this laboratory from the work of several authors. It will be noted that considerable variation occurs between strains of the same species, as well as between different species. For the most part, however, the length of the asexual cycle is 24 hours, or multiples of this figure. The only exceptions are in 3 strains of *P. relictum*, and in *P. gallinaceum* from

fowls; these reports need confirmation. The period of maximum segmentation in different species and strains is exceedingly variable. For example, 3 strains of *P. relictum* with

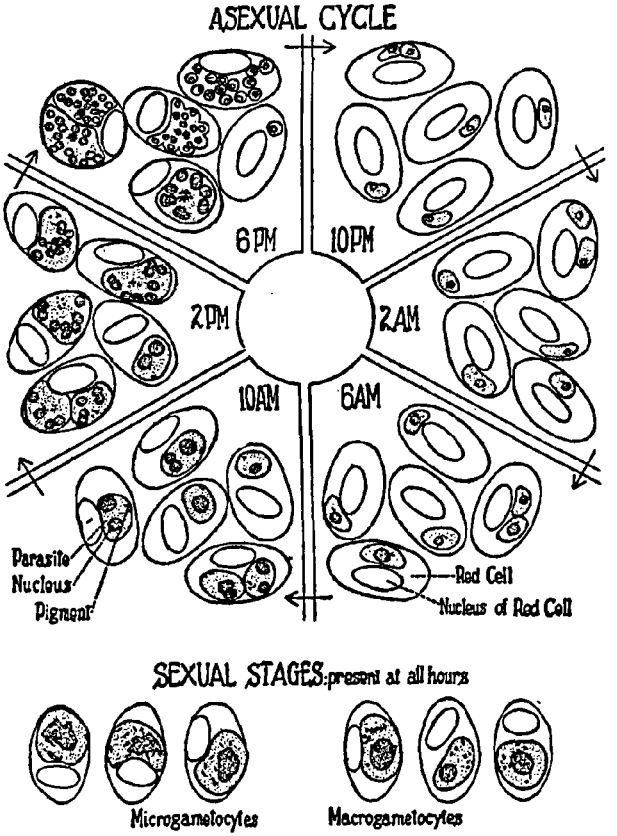


FIGURE 10—Representation of the cycle of reproduction in bird malaria (*P. cathemerium*) showing changes in size (from L. G. Taliaferro, 1928).

matinal segmentation have been described (Huff, 1937; Wolfson, 1937a; Hewitt, 1940b), whereas all other strains of this species which have been studied segment in the evening.

d. *Periodicity in gametocyte production.* It is generally believed that gametocytes originate from asexual parasites although great divergence of opinion exists regarding the

TABLE 10

PERIODIC PHENOMENA IN AVIAN PLASMODIA [1]

Species and strains	Degree of synchronicity	Length of asexual cycle	Segmentation period	Author
P. relictum				
Whitmore strain	low	30 hours	——	L. G. Taliaferro, 1925
Inconstans strain	high	36 hours	12-18 hours	Gingrich, 1929
French strain	low	12 hours	——	Bovet, 1930
Woodthrush strain	very high	24 hours	6-10 A. M.	Wolfson, 1937
P. gallinaceum	very high	36 hours	6 hours (apparently in afternoon)	Giovannola, 1938
P. cathemerium				
Hartman strain	very high	24 hours	6-10 P. M.	L. G. Taliaferro, 1925
Gametocyteless strain	absent	——	——	Huff and Gambrell, 1934
P. circumflexum	low	48 hours	late afternoon	Wolfson, 1936
P. hexamerium	high (2)	48 or 72 (3)	morning	Huff, 1935
P. rouxi	low	24 hours	late morning and early afternoon	Wolfson, 1936
P. nucleophilum	low	24 hours	late morning and early afternoon	Wolfson (unpub.)
	low	24 hours	10 A. M.-5 P. M.	Manwell and Voter, 1939
P. elongatum	very high (2)	24 hours	6-10 A. M.	Huff and Bloom, 1935

Legend:
(—) difficult to interpret.
1 from data compiled by Wolfson (not published).
2 synchronicity supposedly increased by mechanical regulation of light and darkness.
3 segmentation is daily, but Huff believes that the cycle is tertian or quartan.

conditions which bring about the change. Huff (1927) made studies on *P. cathemerium* with regard to changes in the gametocyte number throughout the course of infections. He found that the percentage of gametocytes rose continuously

from the time of their appearance in the peripheral blood, and that the maximum number occurred from one to two days after the maximum number of total parasites. Studying the distribution of the number of gametocytes over a 24-hour period he found no periodicity similar to that found in the asexual forms. Several years later Shah (1934) undertook a study of the periodic development of gametocytes, and observed that although the number of gametocytes increased from day to day with the rise of the infection, the number present in the peripheral blood was not constant at different periods of the day. A periodic development of the gametocytes to maturity was observed from day to day throughout the patent period, and the time when gametocytes become fully grown corresponded with the segmentation of the asexual forms. This work needs further confirmation in infections with other species of bird malaria, but at present it is indicated that the development of gametocytes may follow the development of sexual forms quite closely, in that sexual parasites make their appearance approximately as soon as the asexual parasites, their numerical maxima are coincident, and they develop to maturity at corresponding rates with the asexual forms (figure 11). This has been confirmed in *P. cathemerium* and *P. relictum* infections by Gambrell (1937) (figure 12).

e. *Factors which influence periodicity.* The striking periodic reproduction evidenced by bird malaria parasites has led to many speculations regarding its governing mechanism. Several investigations have been undertaken to determine the nature of the phenomenon, and although a complete picture has never been obtained, several facts of basic importance seem well established. Although the time of segmentation has been artifically altered, it returns to normal within a relatively short time. L. G. Taliaferro (1928), for example, delayed the asexual cycle by refrigerating parasitized blood *in vitro* at 0.5 C. After placing the parasites once more within the body of the host the asexual cycle returned to normal within a comparatively short time, after which it remained normal throughout the rest of the

course of the infection (figure 13). Two explanations for this occurred to the writer; either the asexual cycle repre-

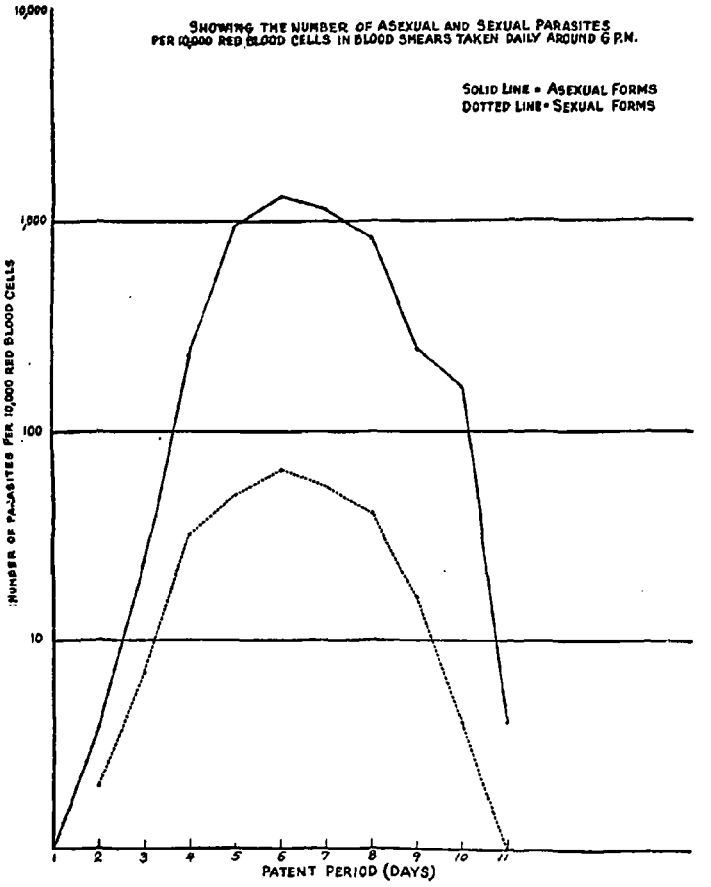


FIGURE 11—Graph showing the number of sexual and asexual parasites throughout the patent period (*P. cathemerium*) (from Shah, 1934).

sents a genotypic character of the parasites which can be temporarily but not permanently changed by environmental factors, or the cycle is forced on the parasites by the activi-

ties of the host or by diurnal changes in the environment. The genetic constitution of malaria parasites has never been

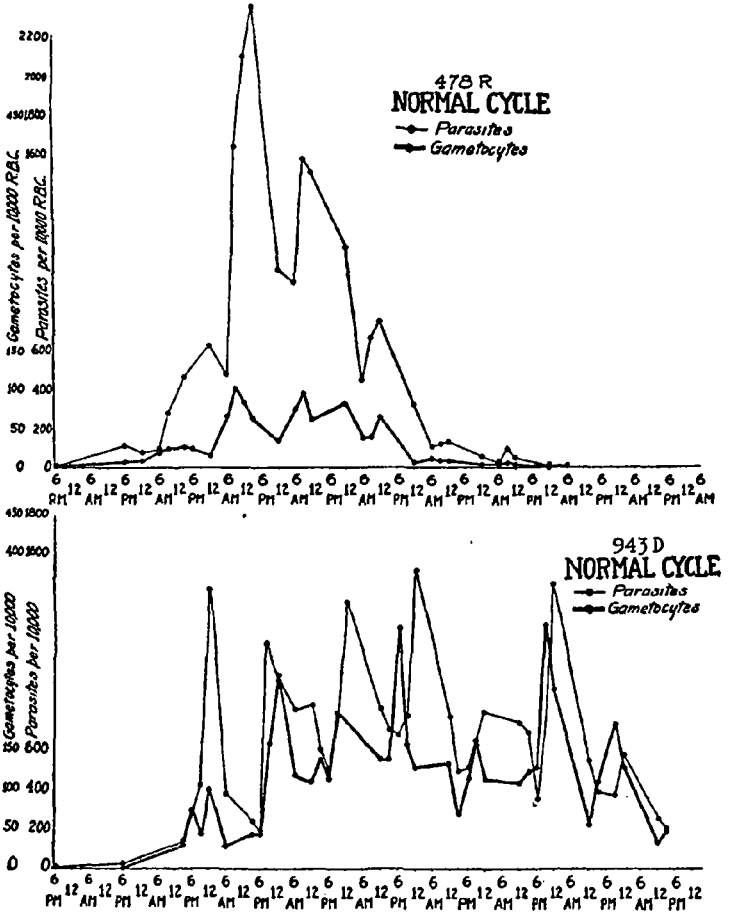


FIGURE 12—Graphs showing the number of sexual and asexual parasites throughout the patent period of infections with *P. relictum* (478R) and *P. cathemerium* (943D) (from Gambrell, 1937).

investigated, and the experimental approach to the problem of periodicity has largely been in the direction of changing the environment or modifying the conditions of the host.

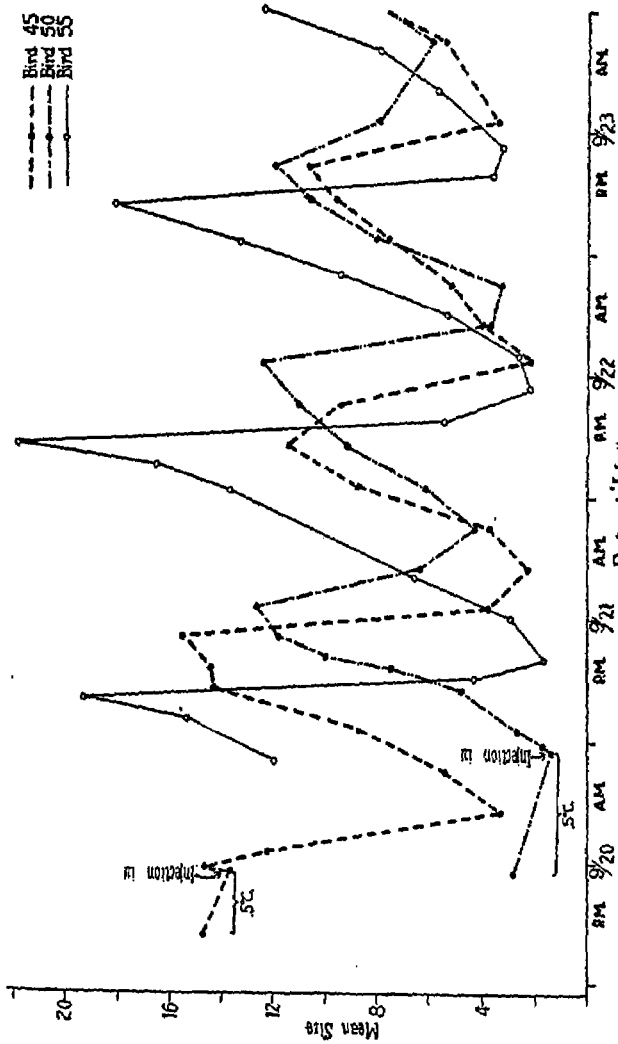


FIGURE 13—Graph showing the cycles of growth and sporulation after refrigeration of parasites (*P. cathemerium*) *in vitro*, and then inoculating them into birds. Bird 55 is a control infection (from L. G. Taliaferro, 1928).

G. H. Boyd (1929) undertook to determine some of the alterations in environmental influences which might possibly affect the asexual cycle. By reversing the light and dark schedule he obtained a reversal of periodicity in the asexual reproduction of *P. cathemerium* without changing its length (figure 14). Gambrell (1937) in similar experiments reversed the time at which peaks of gametocytes appeared. In further studies Boyd found that the feeding habits of the host bear an important relation to the definiteness of the periodicity of reproduction, but that host fatigue or the taking of water play no part. He concluded that the reversals in reproduction which he had previously brought about by changing the normal light and darkness schedule were due to disturbance of the habits of the host rather than to the light itself, and that periodicity is forced on the parasite by some regularly recurring physico-chemical condition in the blood.

Huff and Gambrell (1934) observed that no periodicity (synchronicity?) of reproduction occurred in a gametocyteless strain of *P. cathemerium* which they isolated (figure 28), but do not suggest a reason for this lack of periodic rhythm.

In studies on the mode of action of quinine hydrochloride, G. H. Boyd and Allen (1934) and Lourie (1934c) both report that the drug retards the growth of the parasites and delays the asexual cycle. The characteristic synchronicity of development is also lost during quinine treatment.

Another factor which seems to upset the characteristic periodic rhythm is mentioned by Wolfson (1936d) in her studies on *P. circumflexum.* Following the removal of 350 milligrams of blood from a canary during infection with this species a disturbance in the regularity of reproduction occurred which lasted for a day or two, but then returned to normal.

Stauber (1939) added the effect of temperature to the list of environmental factors which influence periodicity in *P. cathemerium* and *P. relictum* infections. High environmental temperatures alternating with room or ice chest temperatures caused a significant disturbance in the asexual perio-

dicity. The most significant deviation in the cycle occurred when the temperature was high during the young trophozoite stage.

Stauber further found that reversal of the light period was

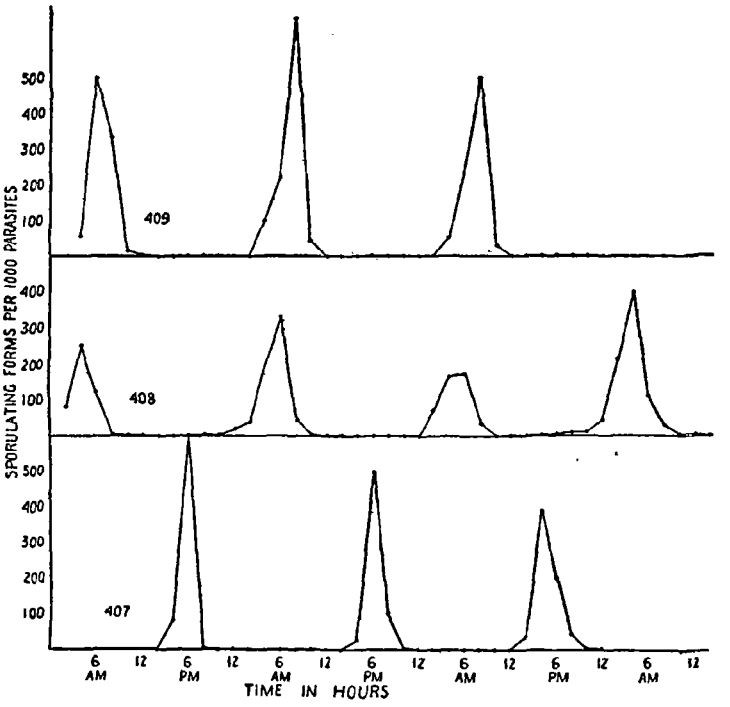


FIGURE 14—Graphs illustrating variations in the reproductive activity of *P. cathemerium* following reversed schedules of activity and rest. Bird 407 is the control infection (from G. H. Boyd, 1929).

active by way of the eyes, but not through the body surface, in affecting periodicity, if the intensity of the change was great enough to cause the host to reverse its periods of activity and sleep. The period of host feeding seemed not to be of importance since segmentation occurred at the end of the light

period regardless of the time food was taken. A diagram of the light-tight cabinet used by Stauber in this work is given in figure 15.

All of the above studies on the asexual cycle, and experimental modifications of the host which affect periodic reproduction have yielded interesting and useful results. It seems

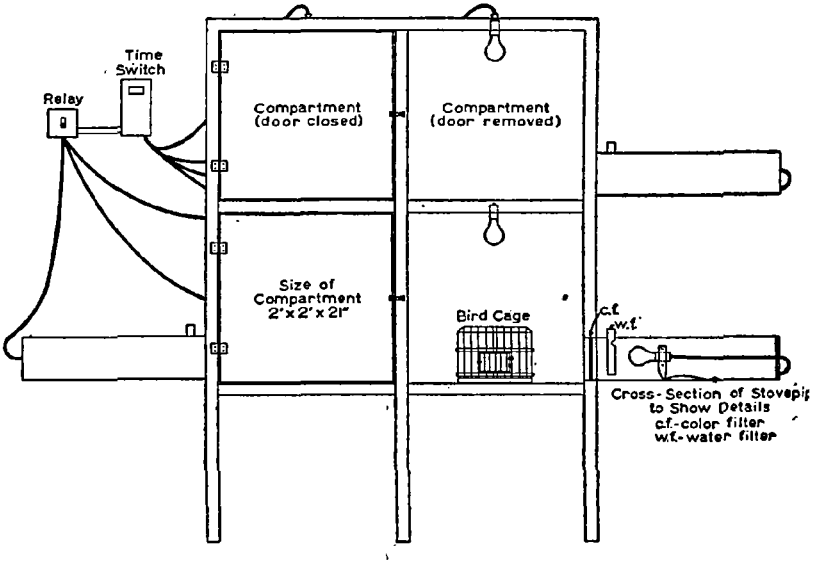


FIGURE 15—Diagram of a light-tight cabinet used in periodicity work (from Stauber, 1939).

clear that no single factor can be asserted at present as the underlying cause for rhythmic reproduction, but that several physico-chemical phenomena in combination with periods of light and darkness of relatively constant length determine the length of the asexual cycle of bird malaria parasites. The genetic constituents of the parasite undoubtedly also play an important part in this respect, but no factual data exist to prove this point.

f. *The mortality of parasites during the asexual cycle.* L. G. Taliaferro (1925) presents data relative to the viability of merozoites during the acute stage of infections with *P. cathemerium,* and concludes that throughout the acute period a small but constant number of merozoites complete their development and reproduce. The average number of merozoites in each mature schizont was said to be 15.5 and during the rise in the parasite curve, 9.98 perished, but 5.05 were able to survive and complete their growth. She believes that the loss of a certain number of merozoites following each sporulation is due to a resistance directed towards the destruction of parasites after they are formed, or that only a small proportion of the merozoites are fitted to live under the conditions presented by the host. Mrs. Taliaferro seemed to believe at the time that destruction of parasites took place largely during the merozoite stage.

Hartman (1927a) later demonstrated that the number of parasites decreased at a constant rate during the period from the entrance of merozoites into red cells until they reached the segmentation stage. Hegner and Hewitt (1938), and Hewitt (1938b) believe that this decline in parasite number during the asexual cycle may be due in part to the fact that many multiple-infected cells occur immediately after segmentation, but that those cells which contain more than 2 or 3 parasites are not able to harbor such a large parasite burden throughout the asexual period (figure 8 and table 9). Consequently parasites within such cells are unable to complete their development.

In a recent investigation of the mortality of merozoites during the asexual cycle by Hegner and Eskridge (1938b) the calculated number of merozoites per segmenter that succeeded in invading red blood cells decreased as the infections progressed, and the total number of parasites decreased greatly during each asexual cycle. A conspicuous mortality of gametocytes was observed in many cases between 6 P. M. and 10 P. M. (*P. cathemerium*); they suggested that this may be due to the breaking down of host cells.

The problem of parasite destruction during each asexual

cycle is probably directly connected with the resistance of the host, but there is evidence to show that it may be due, in whole or in part, to the mechanical destruction of multiple-infected red cells, at least during the period before the crisis. Of interest in the same connection is a paper by G. H. Boyd (1939), bearing on the number of merozoites produced by schizonts of *P. cathemerium* throughout infections, and the rate of destruction of parasites in these infections. He observed that the greatest number of merozoites per schizont occurs in the initial stages of the infection. From an average of 16 ± .1 merozoites per schizont on the first day the number diminished to 12.1 ± .1 merozoites per schizont on the 4th day, and then increased to 14.9 ± .1 on the 7th day (figure 16). The destruction of parasites was comparatively low at the beginning of infections, but after the 3rd day reached 90 per cent, the crisis representing the combined effects of lowered multiplication rates and increased destruction of the parasites by the defense mechanism of the host.

g. *The distribution of parasites throughout the body of the host.* The only species of bird malaria in which segmentation stages have been shown to be definitely localized in the blood of visceral organs is *P. elongatum.* This parasite is capable of living in all blood and hemopoeitic cells of the canary, and segmenters occur more frequently in the bone marrow than in any other part of the body (Huff and Bloom, 1935).

Danilewsky (1889) noted that avian plasmodia could frequently be found in the bone marrow when it was extremely difficult to find them in the peripheral blood. It is not certain, however, that he was dealing with pure infections with *Plasmodium* in these cases, since in some of his birds *Haemoproteus* was also present. A similar situation probably explains Ben-Harel's (1923) report that the bone marrow represents the site of segmentation, since it has been shown that her birds were infected with both *P. relictum* and *P. elongatum.* Neither Hartman (1927a) nor Hegner and Eskridge (1938b) could find definite evidence that *P. relictum* or *P. cathemerium* were localized in the blood of visceral

organs at any time during the asexual cycle. Hewitt (1940a) studied this question in canaries infected with *P. relictum* and *P. cathemerium,* and found that although differences in the distribution of these parasites do occur, they are not constant

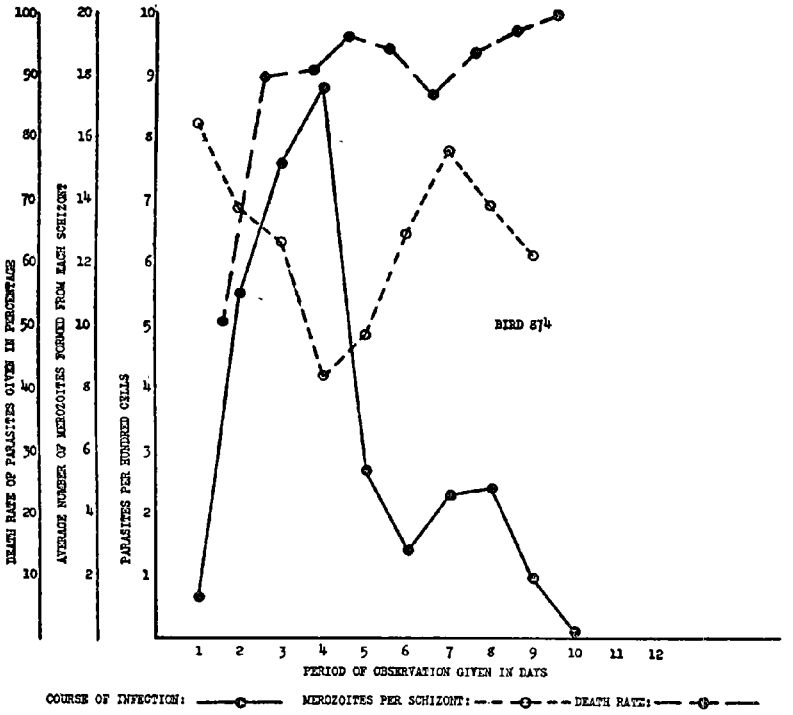


FIGURE 16—Graph showing the rate of parasite destruction and the average number of merozoites produced by each schizont during the course of an infection with *P. cathemerium* (from G. H. Boyd, 1939).

throughout either the patent period or the asexual cycle. All of the organs examined in the several series of experimental birds exhibited a concentration of parasites at one time or another, but outside of the fact that the spleen and bone marrow usually contained fewer parasites than the peripheral blood or liver, considerable variation occurred.

From the few reports available on this subject it appears that of the several species of avian plasmodia which have repeatedly served as experimental laboratory material, the only species which can be compared to *P. falciparum* in man, in that schizogony does not ordinarily occur in the peripheral blood, is *P. elongatum.* Further investigations, however, may show that other species behave similarly.

5. THE BEHAVIOR OF AVIAN PLASMODIA IN ABNORMAL HOSTS

The characteristics of infections in abnormal hosts involve primarily a discussion of the immune mechanism relative to host parasite specificity, but certain features regarding the types of infections produced and the morphology of the parasites in a foreign environment will be considered here. The question of what represents an " abnormal " host must obviously be decided before conclusions can be drawn from experimental inoculations into various species of birds others than that from which the original isolation of the parasite was made. The latter must be regarded, for obvious reasons, as the natural host of the parasite, since the previous history of the organism cannot possibly be known. Koch (1899) successfully inoculated plasmodia from sparrows and goldfinches into canaries, crossbills, and robins, but found pigeons, crows, larks, beechfinches, and titmice to be refractory. Ruge (1901) inoculated canaries with parasites from a sparrow. These two workers were the first to demonstrate that the canary could be used as a convenient laboratory host, and in most of the work which followed, this bird was selected for experimental purposes. The canary is, strictly speaking, an abnormal host for all species of malaria parasites which have been isolated. The great majority of experimental observations have been made from infections in the canary, but it is not certain that the results obtained can be interpreted as being representative of natural infections. However, the comparison standards which have been established for the various species now carried in canaries offset to

a great extent any deviations from the normal which may and probably do occur.

Wasielewski (1908) was able to infect mountain finches, greenfinches, canaries, larks, and goldfinches with a strain of malaria from the chaffinch. It is also of interest that Ross (1898) infected crows and larks with mosquitoes fed on sparrows. None of the workers mentioned, however, give detailed descriptions of the characteristics of the infections produced in the various types of hosts with the same species of parasite. Throughout the years which followed these early experiments a number of species of bird malaria were isolated from various hosts; most of these were readily inoculable to canaries, as well as to other birds in some cases. For a time the canary served its purpose well, but as the knowledge of immunology increased, the need for a larger experimental host was felt. It was not until Brumpt (1936a) imported *P. gallinaceum* in the domestic fowl from Ceylon into France that a large experimental host was made available. This parasite was discovered by Broussais in 1910 in Indo-China, and Brumpt (1935a) named it *P. gallinaceum.* In the following year Brumpt found what he considered to be the same parasite in chickens from Ceylon. *P. lophurae* was discovered by Coggeshall (1938) in the fire-back pheasant, and it was found that this parasite would live in chicks. Coatney (1937) found a strain of *P. relictum* in a mourning dove and a pigeon. Previous attempts to infect chickens with 5 species of avian plasmodia were made by Manwell (1933a) with but little success. He did produce infections showing low parasite levels with *P. relictum, P. circumflexum,* and *P. cathemerium,* however, and his results are of interest since they represent the first real attempt to compare infections in a host which was nearly refractory to infections with the same species commonly carried in canaries.

Manwell inoculated large doses of infective blood into chicks in each case; none of the chicks used were more than 3 weeks old. Blood smears made from infected chicks were sometimes positive after 10 days, but in the majority of

cases parasites could not be demonstrated in the peripheral blood. When parasites were found, their numbers were small, and in most cases only trophozoites occurred. By subinoculation of infected chick blood to canaries it was demonstrated that infections remained at a low level in the chicks for periods ranging from 7 to 10 days. No pathology was exhibited when infected chicks were autopsied.

Herman and Goldfarb (1939) attempted to produce infections with *P. circumflexum* in splenectomized chicks, but found that the removal of the spleen apparently had no influence on the infections, since all attempts were unsuccessful.

Since all previous efforts to infect pigeons with the common species of bird malaria had failed, Wolfson (1937b) attempted to do this with *P. cathemerium*, and also tried to infect a great horned-owl with an inoculum containing *P. relictum* and *P. cathemerium*. Wasielewski (1908) had previously tried unsuccessfully to infect an owl with a species of malaria having round gametocytes.

In the pigeon, Wolfson was able to demonstrate the presence of parasites for 46 days by subinoculation into canaries, and a small number of parasites occurred in the peripheral blood for 5 days after the pigeon was inoculated. Sporulation took place and previously uninfected red cells became infected.

The mixed infection with *P. relictum* and *P. cathemerium* remained in the owl for 10 weeks, thus probably passing through at least 70 asexual generations, and indirect evidence obtained from subinoculations to canaries indicated that the period of segmentation was not reversed because of the nocturnal habits of the host.

In two later papers, Wolfson (1938c, 1939) describes the successful transmission of *P. cathemerium* and *P. relictum* to ducks (*Anas buschas domestica*). Certain morphological and physiological changes in the parasites were noted in this host, as compared with infections in the canary (table 11). In the case of *P. cathemerium* the parasites were more regularly rounded, rather than amoeboid, and more compact. Certain of the gametocytes appeared to have dot-like pigment

granules rather than rod-like granules which are typical of *P. cathemerium* in the canary. From preliminary observations it was observed that the period of segmentation was shifted to a later hour in the evening than normally occurs in *P. cathemerium* infections in canaries. The prepatent period following intravenous inoculation was approximately 3 days. Transmission was accomplished from canary to duck, from

TABLE 11

PARASITE COUNTS IN A DUCK AND A CANARY FOLLOWING INOCULATION WITH *P. cathemerium* (FROM WOLFSON, 1938)

Time	Number of parasites in 10,000 red blood cells	
	Duck 121	Canary 932
First day		
P. M. 3	180	40
P. M. 4	10	40
P. M. 7:30	0	120
Second day		
A. M. 10	0	120
Third day		
A. M. 10	20	840
P. M. 7:30	120	2800
Fourth day		
A. M. 10	120	2900
P. M. 9	240	—
Fifth day		
A. M. 10	580	4880

duck to duck, and from duck to canary. The ducks used for experimental inoculations were about 3 days old.

In discussing morphological differences in *P. relictum* in ducks as compared with canaries, Wolfson (1939) mentions that infections were maintained in ducks for at least 8 months by direct transfer from duck to duck. The gametocytes, instead of being round, appeared elongate in the duck, and the nucleus was surrounded in many cases rather than displaced.

Hegner and West (1940) have recently carried this work further, and have succeeded in subinoculating two strains of *P. cathemerium* into chicks. Parasites have been transferred from chick to chick for 4 transfers, and have been successfully subinoculated into canaries 14 days after the original inoculation. The length of the asexual cycle of *P. cathemerium* in the chick was 48 hours, as compared with 24 hours in canary infections, and the time of segmentation changed from about 7 P. M. to about 9 A. M.

These changes in morphology and physiology suggest that physico-chemical differences may exist between the red cells of canaries, ducks and chickens. They also point to the much more significant fact that the characteristic morphology or physiology of a "good" species may be considerably changed by its introduction into an abnormal host, and that the occurrence of so many species of bird malaria may depend more or less directly on the species of bird from which they were isolated. Further studies along this line are needed, however, before definite conclusions can be drawn.

Brumpt's (1936a) importation of a malaria parasite in the common fowl has solved the need for a large experimental host in the European laboratories, and several American investigators are now carrying *P. lophurae* (Coggeshall, 1938b) in chicks. Brumpt attempted to transmit *P. gallinaceum* to geese, ducks, guinea-fowl, and several other birds, but succeeded in establishing an infection only in the goose, pheasant, partridge, and peacock. Although *P. lophurae* was originally isolated from a fire-back pheasant, it can be successfully carried through chicks for many generations, although frequent transfers are necessary.

The report by Coatney (1937) of *P. relictum* from doves and pigeons, infective to canaries, adds another large experimental host to the growing list. The only investigations thus far carried out with this strain are those of Coatney (1938, 1940). It produced heavy infections in doves and canaries. Active infections have been maintained by frequent passage for 23 days.

CHAPTER VI

SYMPTOMATOLOGY AND PATHOLOGY

As in all other important features of malaria infections in birds it is necessary to go back to papers published by Danilewsky (1885-1890) for the earliest record on symptomatology and pathology. The amazing aptitudes of this thorough and meticulous investigator are probably better expressed in his descriptions of these features of bird malaria infections than in any other one division of the field, since the material which he presented has only been elaborated upon in all succeeding publications without materially changing the essential details. In 1889 Danilewsky stated that the birds which he used in his studies appeared to be in perfect health when they were captured. Many of them were kept for weeks or months in his laboratory, and those which were parasitized could usually not be distinguished externally from unparasitized birds. Out of more than 300 birds which he kept under observation only 4 or 5 died from what he considered to be the effects of the parasite. These birds exhibited an extraordinary increase in parasite number in the peripheral blood. Upon autopsy the spleen and liver were found to be enlarged and contained enormous deposits of pigment. In the spleen the pigment occurred in the form of grains and fragments, both free and in the interior of macrophages. Parasitized cells were also found phagocytized. In infected magpies the spleen sometimes measured 10 mm. wide by 40 mm. long as compared with 5 mm. wide and 22 mm. long in uninfected magpies. A pronounced anaemia was noticed in heavily-infected birds.

In describing symptoms, Danilewsky (1890c) noted that the temperature of infected birds rose moderately (from 1 degree to 1.5 degrees centigrade or over); the birds lost their appetite, became apathetic, their plummage became ruffled, convulsions occurred, and weight was lost. These symp-

toms were found to occur at the time of the sporulation of the parasites. In general, the sickness gradually terminated in from 4 to 6 days, after which recovery followed, parasites disappeared from the blood, and the condition of the bird was once more normal.

In studying the visceral organs as well as the peripheral blood in infected birds Danilewsky further noted that the smallest parasites were found in the immature red cells, as has already been mentioned.

All of the succeeding work on the symptomatology and pathology of bird malaria repeats the essential changes found by Danilewsky, differing only in degree. Kruse (1890) and Labbé (1894) noted similar conditions in birds infected with *Haemoproteus,* and MacCallum (1898a) made a study of the pathological reactions in birds infected with haemocytozoa, including both *Haemoproteus* and *Plasmodium.* MacCallum found the pigment to be scattered throughout the vascular pulp of the spleen, and observed two kinds of pigment within the phagocytic cells, one derived from the haemoglobin of the red cells and the other from the parasite. The endothelial cells lining the capillaries of the liver were greatly swollen with pigment and cellular debris, and the bone marrow, kidneys, intestinal wall, thyroid and adrenals likewise exhibited deposits of pigment. Very little pathology was found in the lungs, stomach, or pancreas. Necroses were observed in several cases, but their dependence upon the presence of malaria parasites was not proven.

Following MacCallum's paper the attention of investigators was turned to problems of immunity and the mosquito transmission of malaria, and further studies on pathology were neglected until 1923 when Ben-Harel reopened the field by presenting data on the pathological changes in canaries infected with what were probably mixed infections with *P. relictum* and *P. cathemerium.* This work was followed by that of Nitsche (1929), the Sergent brothers (1929), Cannon and W. H. Taliaferro (1931), Young (1938), Bloom and W. H. Taliaferro (1938), Hewitt (1939d) and Wolfson (1940a). An account of these find-

ings is presented under the separate headings which follow, together with certain other material reported in various other papers not fundamentally concerned with pathological changes.

1. THE BLOOD

A noticeable fall in red-cell number during the patent period was observed by Ben-Harel (1923), and this is coin-

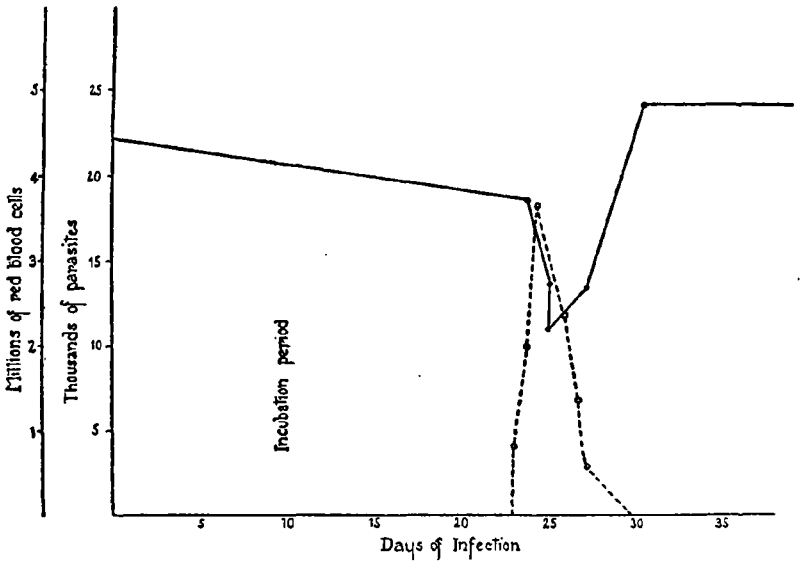


FIGURE 17—Curve showing decrease in number of red cells (solid line) in a malaria-infected bird (from Ben-Harel, 1923).

cident with the rise in parasite number as illustrated in figure 17. A red-cell count as low as 2,500,000 per cmm. was recorded at the crisis of a mixed infection. Young (1938) observed a red-cell count of 2,460,000 per cmm. on the 30th day of a *P. rouxi* infection in canaries. Figure 19 illustrates changes in erythrocyte counts and haemoglobin concentrations in *P. rouxi* infections.

Associated with the fall in red-cell number is an increase in the percentage of reticulated red cells (Hegner and Hewitt, 1937c; Hewitt, 1939a). Just after the crisis in *P. cathemerium* infections, for example, from 50 to 100 per cent of the blood cells may show reticulum when stained supravitally, as shown in figure 18. Furthermore, following

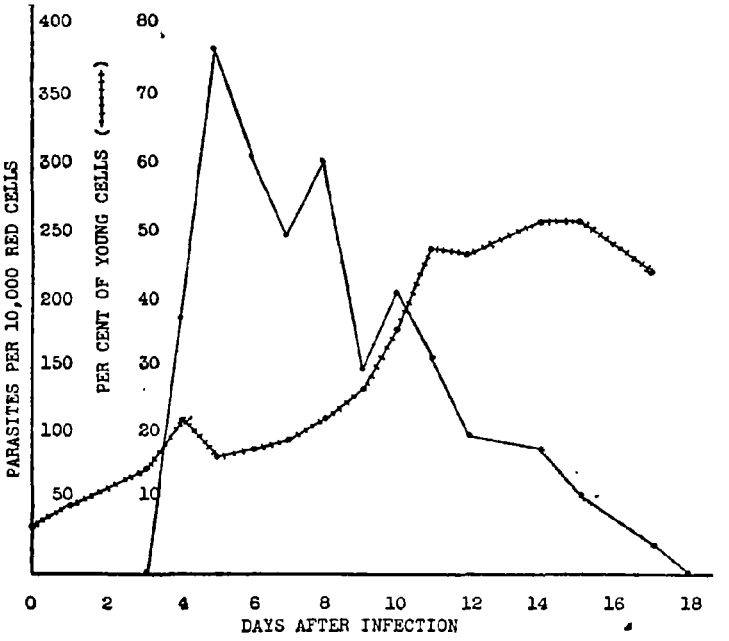


FIGURE 18—Graph showing the increase in young red cells (barred line) during an infection with *P. cathemerium* (from Hegner and Hewitt, 1938).

heavy infections, if the bird does not die, the types of red cells found in the peripheral blood tend to become more and more like the round erythroblasts found in the bone marrow, indicating a tremendous hyperplasia of the latter organ.

Table 12 presents the only published record of the white-blood-cell picture during bird malaria infections (Ben-

Harel, 1923), although it has frequently been observed that macrophages occur in the blood stream during heavy infections. In heavy infections, the serum may become milky or sticky, probably due to the precipitation of globulins. Few birds survive this condition.

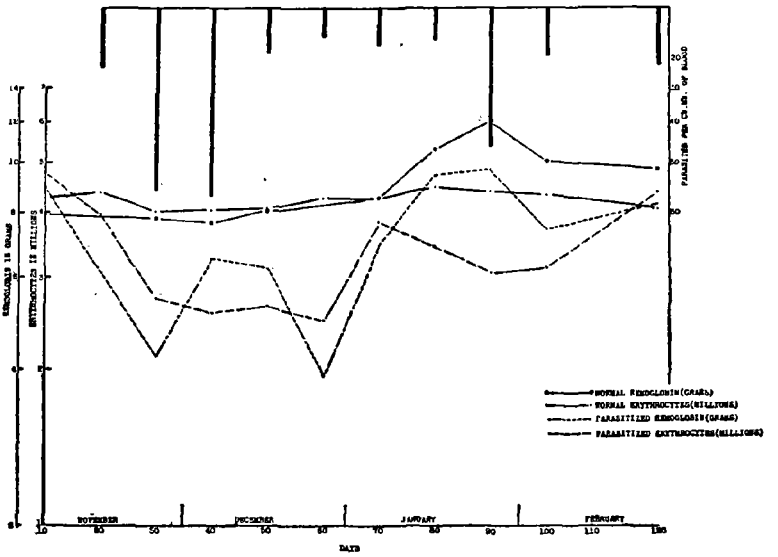


FIGURE 19—Arithlog graph showing changes in haemoglobin concentrations and erythrocyte counts in the blood of normal and *P. rouxi*-infected canaries. The inverted bar diagram represents the number of parasites present at different times during the course of the infection (from Young, 1937).

2. ENLARGEMENT OF THE SPLEEN

A number of authors have given detailed descriptions of splenic enlargement in birds infected with malaria, and for the sake of completeness the observations of Danilewsky (1889) and MacCallum (1898a) in this respect will be repeated. The former author found that the spleens of magpies infected with malaria were frequently enlarged to twice their normal size, this observation being made from natural

infections. MacCallum (1898a) classified spleens as large when they reached 2 mm. by 10 mm., and 3 by 20 mm. (not canaries). Et. Sergent (1920) mentions hypertrophy of the spleen as a very good indication of latent malaria in birds, and states that it is a constant feature of infections. In Ben-Harel's observations (1923) on birds infected with *P. relictum* and *P. elongatum* the maximum size of the spleen recorded is 5 mm. by 15 mm. G. H. Boyd (1925) mentions the fact that in *P. relictum* infections the spleen increases rapidly in size until by the time the peak of the infection is

TABLE 12

DIFFERENTIAL WHITE BLOOD CELL COUNTS DURING A MIXED INFECTION WITH *P. relictum* AND *P. elongatum* (FROM BEN-HAREL, 1923)

	Dec. 15	Dec. 22	Dec. 27	Dec. 28	Jan. 2	Jan. 3	Jan. 5	Jan. 8
Large mononuclear leucocytes.....61%	64%	52%	52%	74%	77.5%	78%	70%	
Lymphocytes 31.5%	20%	30%	32%	11%	13.5%	15%	20%	
Pseudo-polymorphs. 4%	12%	14.5%	14.5%	12.5%	0.9%	5%	7%	
Eosinophiles .....—	—	—	—	—	0.9%	—	2%	
Mast cells 3.5%	4%	3.5%	1.5%	2.5%	0.9%	2%	1%	
Parasites per cmm.. 1	4,900	—	1,908	21,105	13,345	17,835	4,320	

reached it may become from 8 to 10 times its normal size. In a fatal case of *P. rouxi* infection the Sergents and Catanei (1929a) give the spleen size as 3 mm. by 12 mm. The size of the spleen in siskins infected with malaria (species?) is reported by Nitsche (1929) as 6.5 mm. by 15 mm. Cannon and Taliaferro (1931) state that the size of the spleen in *P. cathemerium* infections frequently reaches 5 mm. by 15 mm.; Bloom and Taliaferro (1938) report enlargements in the same species up to 4 mm. by 16 mm.; and Hewitt (1939d) records spleens as large as 9 mm. by 25 mm. in *P. cathemerium* infections. A table giving the spleen weights (in milligrams) of malarious and non-malarious birds is presented by Manwell (1932) from infections with *P. elon-*

gatum, P. rouxi, P. relictum, and *P. cathemerium,* in both acute and chronic cases. These data are reproduced in table 13. Young (1938) found the maximum size of the spleen in *P. rouxi* infections to be 5 mm. by 15 mm.

TABLE 13

SPLEEN WEIGHTS IN CANARIES INFECTED WITH FOUR SPECIES OF BIRD MALARIA AND IN NON-INFECTED CONTROLS (FROM MANWELL, 1932)

	WEEKS SINCE INFECTION (MEAN)	WEIGHT GROUPS (IN MGM.)									MEAN
		1-10	11-20	21-30	31-40	41-50	51-60	61-80	81-100	Over 100	
		a. *Plasmodium elongatum*									
Acute cases (5)....	2.2	..	..	2	1	..	..	..	..	2	82.6
Chronic cases (9)..	14.0	2	1	..	1	2	..	1	..	2	69.1
		b. *Plasmodium rouxi*									
Acute cases (9)....	3.1	1	1	1	3	1	1	1	..	..	35.9
Chronic cases (11).	17.4	..	2	2	1	2	1	1	2	..	48.1
		c. *Plasmodium praecox*									
Acute cases (1)....	2	..	..	..	..	1	..	..	..	..	82.6
Chronic cases (5)..	25.6	1	..	2	..	1	1	..	..	..	32.0
		d. *Plasmodium cathemerium*									
Acute cases (0)....	....	..	..	..	..	..	..	..	..	..	....
Chronic cases (2)..	15	..	..	..	1	1	..	..	..	..	40.5
		e. Malaria-free birds									
Total cases (22)...	....	4	3	4	3	4	1	..	3	..	36.2

Note: All cases which had been infected for four weeks or less were considered to be acute; if infected for more than four weeks they were regarded as chronic.

It is apparent from the variations in splenic hypertrophy reported by different investigators for the same species and for different species that no general statement can be made with respect to the spleen size which will be reached in any single infection with a particular species. Manwell (1932) says, "It is clear however that splenic enlargement is in

itself a very unreliable sign of malaria, for a few birds which were unquestionably infected and even showing a considerable number of parasites were found to have spleens weighing 10 mgms. or less, while several birds known to be malaria-free both from previous history and blood examination, had spleens weighing 80 to 100 mgms. . . . The only point which it is desired to make is that splenomegaly in birds is not the certain sign of malaria which it has been claimed to be, and that some birds may have very small spleens and yet have malarial parasites easily demonstrable in the blood." Hewitt (1939d), furthermore, found no correlation between either the number of parasites or the duration of the infection and the size of the spleen in *P. cathemerium* infections, indicating that individual variations in each host occur relative to this point. Figure 20 illustrates the progressive enlargement of the spleen encountered in infections with this parasite, and plate IX illustrates an unusually large spleen from a canary infected with another strain of *P. cathemerium.*

W. H. Taliaferro and Mulligan (1937) review the factors which appear to be responsible for the enlargement of the spleen in the various species of malaria parasites. They state that the primary enlargement is due to simple hyperaemia which is replaced or augmented by an increase in the cellular elements of the pulp and possibly also by an increase in the fibrous tissue. The highest degree of enlargement is reached, in their opinion, at the time of the crisis, after which it may continue to remain high for a time but eventually subsides as latency ensues. They enumerate various exceptions to the process, including individual variations due to both the species of animal and strain of the parasite.

3. SPLENIC INFARCTION

The occurrence of splenic infarcts in birds infected with *P. cathemerium* is reported by Bloom and W. H. Taliaferro (1938), Hewitt (1939d), and Wolfson (1940a). Bloom and Taliaferro found that infarcts occurred sporadically in

the spleens of their infected canaries and an account is given of the rapid regeneration of these infarcts. Hewitt observed infarcts of various types (see figure 20) in 51 (47 per cent) of 107 canaries infected with a virulent strain of *P. cathemerium*. They occurred as early as the third day after inocu-

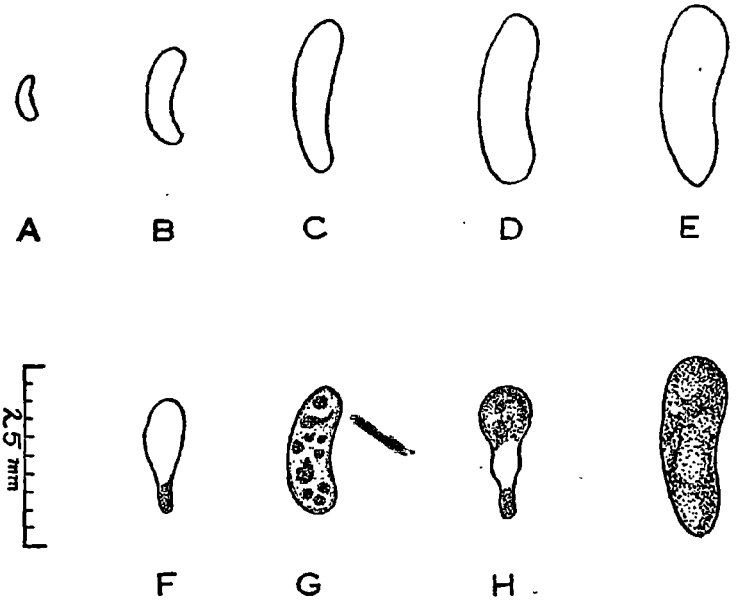


FIGURE 20—Drawings showing enlarged canary spleens (B to E) and infarcted spleens (F to I) from infections with a virulent strain of *P. cathemerium*. A represents the size of the normal canary spleen (drawn to scale) (from Hewitt, 1939).

lation and throughout the course of the patent period. The production of these infarcts seemed not to depend on the number of parasites present at the time infarction occurred, nor upon the size of the spleen or the amount of inoculum, and they occurred at different times during infections in different series of birds. Large thrombi in the central vein of the spleen were always associated with infarcts, and

numerous smaller thrombi occurred in the arterioles and venules. Some of the large thrombi were followed in serial sections and were found to involve a large portion of the central vein. Regeneration and repair, similar to that observed by Bloom and Taliaferro, were noted.

4. CELLULAR REACTIONS

The most complete account of the cellular reactions in the spleen and liver of infected birds is that of Cannon and Taliaferro (1931). Ben-Harel (1923) and Nitsche (1929) reported similar changes earlier, but less in detail, and Young (1938) compared Cannon and Taliaferro's observations in *P. cathemerium* infections with those he made in *P. rouxi* infections, finding the same sort of response but to a lesser degree. The most conspicuous changes in the spleen, coincident with its enlargement and hyperplasia, are the following (in *P. cathemerium* and *P. rouxi* infections):

(1). The sinuses and capillaries become dilated and engorged with all kinds of blood cells.

(2). Pigment is deposited in large quantities and is rapidly taken up by the phagocytic cells.

(3). Mitotic figures become increasingly common in white cells in the follicles as the infection progresses.

(4). The lymphoid tissue is greatly increased.

(5). Thrombi and infarcts may bring about large areas of necrosis.

The cellular reactions in the liver are similar to those in the spleen, in that the endothelial cells lining the capillaries become swollen with pigment, the sinuses become enlarged and engorged with lymphoid cells, and mitotic figures increase in number as the infection progresses. The Kupffer cells are active in engulfing pigment and parasitized red cells (figure 22, C.). Focal necroses occur, but these are infrequent. The hepatic cells may become vacuolated, and frequently show fatty degenerative changes. Extramedullary haemo-

poeisis is indicated by an increase of eosinophilic crystalloid cells, and the liver cords may become strikingly disoriented.

Partial or complete destruction of the spleen by infarcts stimulates more marked changes in the liver than occur ordinarily. Hewitt (1939d) found that in every case where splenic infarcts occurred the liver was markedly more hyperplastic than in birds where the spleen was not infarcted, indicating a compensation on the part of the former organ for the destruction of large amounts of splenic tissue.

5. THE BONE MARROW AND OTHER VISCERAL ORGANS

Very little information has been published concerning the changes which take place in the bone marrow. Compared to the spleen and liver little pigment deposition takes place in the bone marrow, and phagocytosis, although present, is not marked. As infections progress the ratio of fat to haemopoeitic tissue changes considerably, and hyperplasia becomes extremely pronounced. Heavy infections of long standing may bring about aplastic marrow, and calcareous infiltration.

In the brain, the capillaries may appear to be plugged with parasitized cells, and local haemorrhages and oedema occurs in some instances (Wolfson, 1940a). No prominent pathological changes are known to take place in the other visceral organs, other than deposition of pigment and phagocytic activity.

6. THE RELATION BETWEEN INFECTIONS AND BODY TEMPERATURE

Huff (1939a) has recently described temperature changes in canaries infected with the M strain of *P. cathemerium* and the R and G strains of *P. relictum* (figure 21). This is the only published account of its kind in the field (other than the brief mention of body temperature changes made by Danilewsky and already mentioned) and properly belongs in a discussion of symptoms and pathology.

Temperature readings were taken by inserting hypodermic thermocouples into the pectoral muscles of experimental

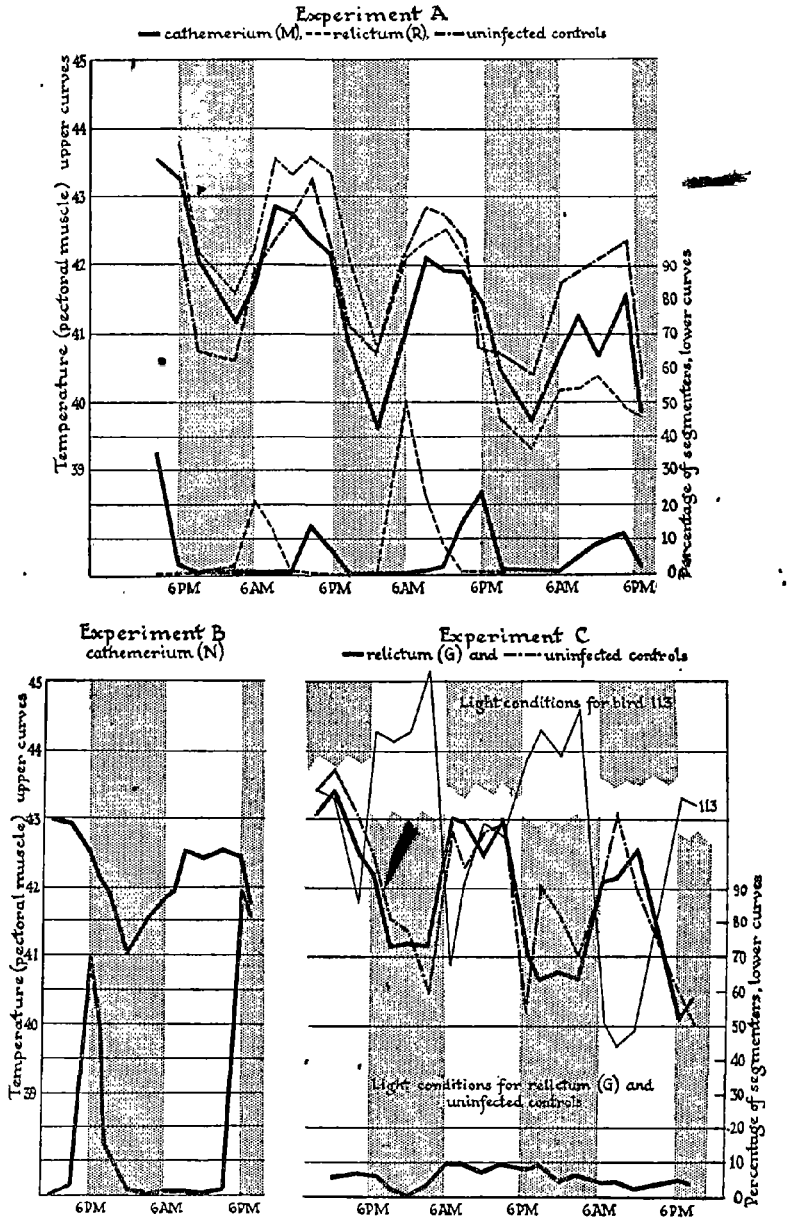


FIGURE 21—Graphs of temperatures obtained by thermocouple readings on pectoral muscles of normal canaries and canaries infected with 4 different strains of *Plasmodium*. The shaded and unshaded areas represent periods of darkness and light (from Huff, 1939).

birds. It was found that no temperature rises accompanied the periods of greatest segmentation of the asexual forms, such as are characteristic of human malaria. Since the temperature curves for infected birds apparently did not depart from the temperature curves of normal birds it appears that the bird malaria parasites do not behave as pyrogenic agents. The mean day temperature for uninfected birds was established as 42.3° C. ± 0.05 and the mean night temperature as 41.0° C. ± 0.26. Significant variations in the temperatures of uninfected birds occurred in certain individuals and in one case a temperature of 45.3° C. was recorded in an apparently healthy animal. A rise in temperature occurred immediately after awakening and was considerably higher after birds had been allowed to fly for 35 feet. The author points out that although fluctuations in avian temperatures through a 24-hour period have been known to exist for some time, his experiments give a more accurate picture of the body temperatures of birds than has been done previously, and also establishes a normal temperature range for canaries. More work of a similar nature is necessary before it can be definitely established that temperature rises do not accompany infections with other species and strains of avian plasmodia.

CHAPTER VII

IMMUNE REACTIONS

The problems of immunity in bird malaria are extremely varied and complex. Natural immunity, specific immunity, recriprocal or cross immunity, immunity to superinfection, strain immunity, passive immunity, active immunity, relative immunity, humeral antibodies, phagocytosis, melanoflocculation, vaccination, and relapse phenomena have all been studied.

One of the earliest stimuli towards research with the avian plasmodia was the belief that the experimental results obtained could be directly applied to human malaria. Investigators immediately chose the two most obvious lines of attack which would probably have some application to the disease in human beings, namely immunity and chemotherapy. The bulk of research in the field has consequently been done in these two subdivisions of the general subject, with the result, as predicted by the early workers, that much of our knowledge concerning immune responses and therapeusis in human malaria has been derived from comparable studies on birds. Of the two, perhaps the immunological relationships in human and bird malaria are more likely to show differences when further material is forthcoming from inoculation malaria in syphilitics, but there can be little doubt that the mechanism of defense in both the human and avian types of the disease is strikingly similar.

1. PHAGOCYTOSIS

Chronologically, the role of the phagocytic cells in the bodily defense against malaria parasites was the first immunological subject to be investigated in birds. This was natural, because the discovery of both human and bird malaria was coincident with the researches of Metchnikoff

and his colleagues at the Pasteur Institute in Paris. Danilewsky's description (1890b) of phagocytosis in bird malaria is as follows: "Some of the parasites eventually succumb in the conflict between the phagocytic activity of certain cells, while others conserve their vitality and in so doing are able to develop to maturity. It is very probable that the same conflict occurs in the malarial infection in man, above all in the spleen and bone marrow. We may consider these organs porous filters, in which the blood leaves foreign bodies in passing, and the parasites are actively engulfed by the protoplasmic elements."

Less importance was given to the phagocytes by Labbé (1894); he states that, "the infection is subject to certain conditions of immunity which depend upon the intensity of the infection and on the species of host involved; phagocytosis, as a method of defense against the parasites, is not generally important, but in certain cases the leucocytes do acquire the phagocytic power."

MacCallum (1898a) and Ben-Harel (1923) studied the reactions of the spleen, liver and bone-marrow during infections and found that the phagocytes in these organs actively engulfed parasitized red cells and pigment. Ben-Harel (1923) concluded that the great reduction in the parasite number following the crisis was due to their destruction by fixed-tissue cells and circulating phagocytes.

The most thorough investigation of the cellular basis of immunity, however, is the work of Cannon and W. H. Taliaferro (1931) on infections with *P. cathemerium.* This species of parasite was chosen because its asexual cycle was better understood than that of any other known species, and because it exhibited regular periodicity. Both primary infections and superinfections were studied. They state that during the general course of bird malaria infections the parasites accumulate rapidly in the blood until the crisis when the maximum number is reached, after which a sharp drop in number occurs and the parasites rapidly disappear from the peripheral blood. This, they believe, is brought by the antagonism of two active processes which occur simultane-

ously: (1) the reproduction and development of the parasites, and (2) their ingestion by phagocytes. (A sharp drop in parasite number following the maximum number of parasites is, however, the exception rather than the rule, as will be shown later).

In primary infections phagocytosis by macrophages in the spleen and liver was observed by these writers to a slight extent as early as 4 hours following the introduction of parasites into the blood stream. Thereafter it occurred constantly and in progressively increasingly amounts throughout the entire acute period. The height of activation of the phagocytic cells appeared between the 8th and 10th days of the infections, coincident with the crisis, " when the parasites disappear precipitately from the peripheral circulation."

In latent birds inoculated intravenously with parasites (superinfection), phagocytosis occurred much more rapidly and effectively than in birds infected for the first time. Phagocytosis during superinfection was well marked within 15 minutes after the introduction of parasites, and within 24 to 48 hours successfully removed the parasites.

As pointed out in a further discussion by W. H. Taliaferro and Mulligan (1937) of malaria in general and of monkey malaria in particular, the parasites are subjected to adverse conditions as soon as they enter the host. The antiparasitic factors which are operative prior to the crisis of the infection and which provoke a limited degree of phagocytic activity constitute *natural resistance.* Antiparasitic factors which become effective at the crisis, however, and which thereafter keep the infection at a low level may be classified as *acquired resistance* since they occur only in the immune host and may be demonstrated by the response of superinfected birds.

The cellular basis of immunity in bird malaria has also been studied by Kritschewski and Meerson (1932), who likewise showed that the reticulo-endothelial system is chiefly responsible for the destruction of parasites, and that the action of this system is specific. One of the chief premises upon which the theory is based, however, lies in the supposition that the number of parasites which survive in each

asexual cycle is constant during the acute rise of the infection, or during the disappearance of parasites in superinfected birds, as described by L. G. Taliaferro (1925), Hartman (1927a) and Lourie (1934c). It has been shown by G. H. Boyd and Allen (1934), and G. H. Boyd (1939) that fluctuations occur in the number of merozoites produced during the course of infection with *P. cathemerium,* particularly during the acute rise and coincident with the crisis. Furthermore, a precipitous drop in the number of parasites immediately following the crisis is not a constant feature of bird malaria infections (figures 3 and 4). It is therefore by no means conclusively established that acquired immunity is developed suddenly, but it seems more likely that phagocytosis, in combination with other immune mechanisms, increases in degree throughout the acute rise of infections and continues to rid the host of parasites after the parasite peak has been reached. Phagocytes apparently become active as soon as malaria parasites are introduced into the host, and increase in their activity as the infections progress until a point is reached when they reduce the parasite number sufficiently to cause a drop in the infection curve.

Figure 22 illustrates various types of phagocytic cells containing engulfed pigment and parasitized cells.

2. IMMUNITY TO SUPERINFECTION

Another characteristic of malaria infections in birds that was early recognized is the fact that infected birds are immune to reinfection only if complete recovery has occurred and all of the parasites from the first infection have disappeared. Wasielewski (1901) is usually credited with being the first to show that malaria in birds runs a chronic course, and that parasites can be demonstrated in the blood for a long time, although Danilewsky (1889) remarked 12 years previously that he kept infected wild birds in his laboratory for weeks and even months while following the development of the parasites (*Plasmodium* and *Haemoproteus*). Previous to Wasielewski's observation it was believed (Koch, 1899; Ruge, 1901) that bird malaria was a self-limiting infec-

tion, with rapid and complete recovery followed by immunity to superinfection. Moldovan (1912) demonstrated that recovered birds were susceptible to superinfection, but that

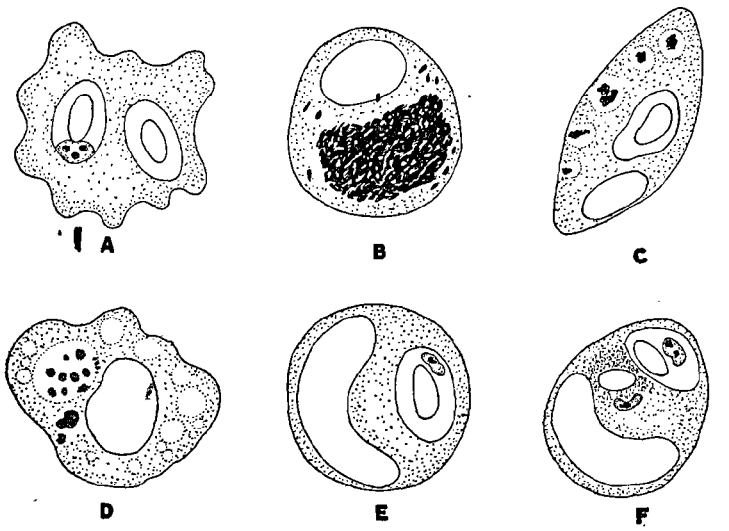


FIGURE 22—Drawings of phagocytic cells containing malaria pigment and parasitized red cells (x 1500).

A. An amoeboid phagocyte containing a parasitized blood cell and an unparasitized blood cell (after Danilewsky, 1895).

B. A phagocytic cell from the spleen, containing a mass of malarial pigment (after MacCallum, 1898).

C. A Kupffer cell from the liver, containing 5 masses of engulfed malarial pigment and a disintegrating unparasitized red cell (after Cannon and Taliaferro, 1935).

D. An amoeboid phagocyte from the bone marrow, containing pigment and a disintegrating segmenter (original).

E. A monocyte from the spleen, containing a parasitized red cell (original).

F. A monocyte from the spleen, containing a parasitized red cell and a disintegrating parasitized red cell (original).

chronically infected birds could not be successfully inoculated with the same species of parasite. This situation has come to be known as "immunity to superinfection," and is supported by the work of Whitmore (1918), Et. Sergent and Hempl (1917), Kikuth and Tropp (1917), Mazza (1924),

W. H. and L. G. Taliaferro (1929), Lotze (1930), Manwell (1934b), and many others. Figure 23 graphically illustrates the rapid disappearance of parasitized cells which were inoculated into a latent case with the same species of parasite (from W. H., and L. G. Taliaferro, 1929a). Manwell (1938a) remarks that the strain of malaria with which Moldovan worked must have been rather exceptional in its

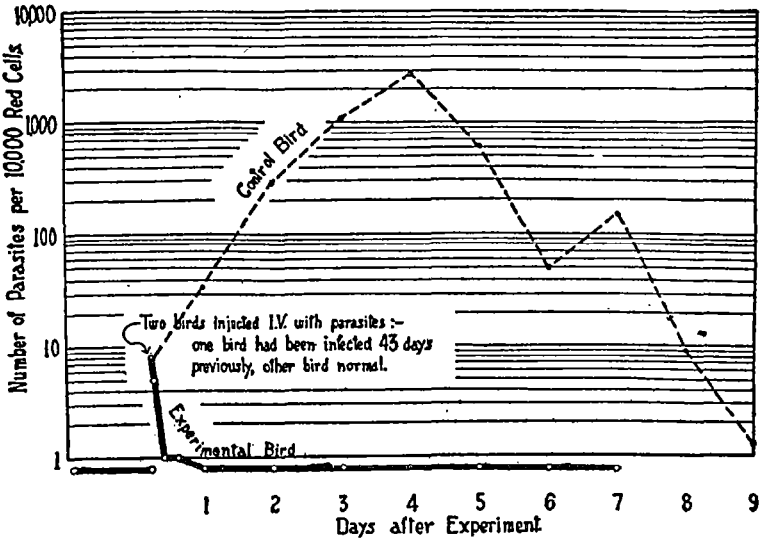


FIGURE 23—Graph showing the disappearance of washed parasitized cells after injection into the blood of a bird with a latent infection with the same species of parasite (*P. cathemerium*) (from W. H. and L. G. Taliaferro, 1929).

tendency to natural recovery, although Mazza (1924) and Gingrich (1932), as well as Manwell, and Huff (quoted by Manwell, 1938a) have observed natural recovery in a few instances, particularly with regard to infections with *P. elongatum*. It is possible that other species and strains differ in this respect. W. H., and L. G. Taliaferro (1929) report a high degree of immunity to superinfection in *P. cathemerium* infections as long as 656 days after the primary infection.

The duration of malaria infections in birds has been observed by several investigators. Et. Sergent and Hempl (1917) state that 4 out of 5 birds which had been infected for at least 2½ years were still infected. Whitmore (1918) reports several cases of similar duration, and Mazza (1923) found one bird to be infected 4 years after the initial infection. The Sergents and Catanei (1928) demonstrated *P. rouxi* in birds inoculated 3 years and 8 months before, and Manwell (1929, 1934b) showed that parasites persist in the body of the host in a few instances for as long as 3 years. A recent report by Bishop, Tate and Thorpe (1938) records the presence of *P. relictum* in a bird 8 years and 3 months after the original infection.

These records are of fundamental importance in testing the effect of certain chemical agents on the avian plasmodia, since naturally recovery is apparently rare.

Much of our understanding of the various immune mechanisms operative in infected birds has resulted from studies of immunity to superinfection. Cannon and Taliaferro's (1931) work in this connection has already been mentioned, and the previous work of W. H., and L. G. Taliaferro (1929) on humeral antibodies is also based largely on superinfections. The Sergents and Catanei (1934), and Sergent, Parrot and Donatien (1924) have even suggested a new name for this type of immunity, which is demonstrable only when parasites are present in the body of the host and disappears when complete recovery occurs. They use the word "premunition" to differentiate the phenomenon from the lasting immunity characterized by some of the other protozoa, bacteria, and filterable viruses. In man, *P. malariae* has been reported up to 30 years (Manwell, 1934b) and 40 years (Jankelson, 1931) after the date of the original infection.

3. RECIPROCAL OR CROSS IMMUNITY

The cross immune relationships between various species and strains of avian plasmodia is a field of investigation which has thrown light on the species problem as well as the host

defense mechanism. The factors governing cross immunity are probably the same as those which bring about immunity to superinfection, although just what all of these factors are is not yet clear. Two closely related types of studies have been made; one is called *recriprocal species immunity,* in which different species are inoculated one against the other, and *recriprocal strain immunity,* involving different strains of the same species. The procedure used in making crosses is to select latent cases which are not suffering from a parasite relapse, and to inoculate them with a second species or strain of malaria. Manwell (1938a) has found that intramuscular inoculations give a more sensitive measure of reciprocal immunity than intravenous inoculations. After the cross inoculation has been made, the blood is examined for a period of several weeks or months and the degree of the superimposed infection is compared with the usual course of an uncomplicated primary infection. One self-evident difficulty that the investigator encounters is that of separating two types of plasmodia which may occur in the blood stream after cross inoculations have been made. With the smaller species (*P. vaughani, P. rouxi,* etc.) this presents a particularly difficult problem and the degree of immunity is not easy to estimate in such cases.

Cross-immunity studies have been made by Hartman (1927a), Kikuth (1931), the Sergents and Catanei (1931b, 1932), Gingrich (1932), Manwell (1934d, 1936c, 1938a), Manwell and Goldstein (1938a and c, 1939c), Wolfson (1938a), and Redmond (1939a). Table 14 (from Manwell, 1938a) shows the degree of reciprocal species immunity exhibited by 9 species of bird malaria as reported by various authors mentioned above, and in table 15 are presented Manwell and Goldstein's (1939c) data on reciprocal strain immunity shown in 6 strains of *P. circumflexum.*

A number of important conclusions have been drawn from the results obtained in cross immunity experiments. In the first place, the work thus far reported is all in agreement in that certain species of bird malaria confer a certain degree of immunity to reinfection with some species and not with

TABLE 14

IMMUNITY TO CROSS-INFECTION AND SUPERINFECTION EXHIBITED BY VARIOUS SPECIES OF BIRD MALARIA *
(From Manwell, 1938)

Chronic infections Species:	Active infections: *cathemerium*	*praecox* (*relictum*)	*circumflexum*	*elongatum*	*hexamerium*	*nucleophilum*	*polare*	*rouxi*	*vaughani*
cathemerium	+++ [1,3] †	++ [1,5,6] — [2]	+++ [3]	— [1,2,3]	+ 7(4)	+ 6(3)		++ — [1]	+ ? 6(5)
praecox (*relictum*)	+++ [1] — [2,3] ++ [5,6]	+++ [1,4]	+++ [3]	— [1,2,3,4,7]	+ 6(4)	+ 13(9)		— [1]	+ ? 5(3)
circumflexum	+++ [3]	+++ [3]	+++ [3]	— [3]	+ ? 7(6)	+ 7(4)	+ ? 6(4)		+ ? 6(7)
elongatum	— [1,2]	— [1,2,3]	— [3]	+++ [1,7]	— 6(4)	— 6(6)		— [1]	— 6(3)
hexamerium	+ 8(3)	— 6(3)	+ 6(2)	— 6(4)	++ 4(2)	++ 6(2)		+ 6(2)	+ 3(2)
nucleophilum	++ 10(3)	— 9(5)	+ ? 6(3)	— 6(2)	+ 6(4)	++ 6(6)	+ 4(2)	— 4(1)	+ 6(5)
polare					— 3(2)	— 3(2)			
rouxi	++ — [1] — [6]	— [1,6]		— [1]	++ 6(3)	+ 9(8)		++ [1]	++ 3(1)
vaughani	++ 7(2)	— 6(2)	+ ? 6(3)	— 6(4)	+ 6(4)	++ 7(5)		+ 4(6)	++ 6(3)

[1] Gingrich, 1932. [2] Hartman, 1927a. [3] Kikuth, 1931. [4] Manwell, 1929. [5] Manwell, 1936a. [6] Sergent, Ed. and Et. and A. Catanei, 1931. [7] Sergent, Ed. and Et. and A. Catanei, 1932.

* Numbers not bracketed refer to the number of chronic infections used; numbers in brackets refer to the number of controls used. The number of intravenously and intramuscularly inoculated birds was about equal. Totals: crossed infections 278; controls 157 = 435 (total).
† +++ = strong immunity. ++ = moderate immunity. + = slight immunity. — = no immunity.

others. A latent infection with *P. cathemerium,* for example, will confer partial cross immunity to *P. relictum* and *P. rouxi,* and no cross immunity to *P. elongatum* (Gingrich, 1932). Infection with *P. elongatum,* on the other hand, does not protect against any other species, nor do any of them confer immunity to *P. elongatum* (Manwell, 1938a). Infection with any of the smaller species (*P. hexamerium, P. vaughani, P. rouxi,* and *P. nucleophilum*) confers protection against any of the other three, but the degree of reciprocal immunity is generally not very strong (Manwell, 1938a). Different strains of *P. circumflexum* isolated by Manwell and Goldstein (1939c) exhibited strong mutual protection, although in general to a lesser degree than when superinfection was done with a homologous strain. Redmond (1939a) finds that the virulence of different strains of *P. relictum* and *P. cathemerium* is correlated with the immune reactions of the same strains, the most virulent strains producing the greatest degrees of protection against superinfection with other strains.

Any interpretation given to these various phases of cross immunity phenomena must naturally be complex, and at present purely theoretical. The use of cross immunity tests to determine the specificity of malaria parasites must be approached with the reservation in mind that reciprocal immunity cannot be regarded as an essential criterion for species differentiation (Manwell, 1938b). When no reciprocal immunity exists, however, there is still useful evidence of specificity. The relationship between species might in the future be better established when more data are available concerning their reciprocal immunity. The fact that *P. elongatum,* for example, does not produce cross immunity to any other species yet tried, coupled with the findings of Huff. and Bloom (1935) regarding its occurrence in white blood cells as well as in red blood cells, strongly suggests that this parasite is quite widely separated physiologically from all other known species of bird malaria. Finally, the

strain immunity exhibited by *P. relictum, P. cathemerium,* and *P. circumflexum* (Redmond, 1939a; Manwell and Goldstein, 1939c) is similar in many respects to the strain immunity known to occur in human malaria, and further investigations with the bird malaria parasites may help to clear up some of the perplexities encountered in the immunology of the human disease.

Wolfson and Causey (1939) have recently demonstrated that immunity to superinfection in two strains of *P. cathemerium* is effective when the strains are crossed by mosquito inoculation.

A discussion of cross immune reactions in monkey malaria and in human malaria may be found in papers by Mulligan and Sinton (1933), M. Boyd and Kitchen (1937), and Mayne and Young (1938).

4. PASSIVE IMMUNITY

The passage of blood constituents from a previously infected host to a susceptible host has been tried a number of times in attempts to demonstrate the presence of humeral antibodies in bird malaria, but the results so far achieved are remarkably variable. Moldovan (1912) left serum of birds with latent infections in contact with parasitized cells for 1 hour at 37° C. but no protection was demonstrable when these parasites were inoculated into birds. W. H., and L. G. Taliaferro (1929) were unable to demonstrate either protective or curative action when latent serum was injected into parasitized birds, and latent serum did not sensitize parasites *in vitro.* They thus concluded that no humeral antibody is involved in the acquired immunity of birds with latent infections to superinfection.

The Sergents (1922b) attempted to determine whether fresh splenic extracts prepared from canaries and mice would have a preventive or curative effect on infections with *P. relictum,* but achieved negative results in nearly every case. The few instances of possible protection which they did observe are so variable that definite conclusions cannot be

TABLE 15

RECIPROCAL IMMUNITY EXHIBITED BY VARIOUS STRAINS OF *Plasmodium circumflexum*
(FROM MANWELL AND GOLDSTEIN, 1939)

Chronic infections Strains	Degree of immunity	*A* Experimental cases	*A* Controls	Degree of immunity	*B* Experimental cases	*B* Controls	Degree of immunity	*C* Experimental cases	*C* Controls	Degree of immunity	*D* Experimental cases	*D* Controls	Degree of immunity	*E* Experimental cases	*E* Controls	Degree of immunity	*G* Experimental cases	*G* Controls
A	++++	5	7	++++	4	4	++++	4	4	++++	4	6	+	7	10	++++	4	5
B	++++	6	7	++++	4	4	++++	4	4	++++	2	3	+	7	10	++	4	5
C	++++	8	9	++++	4	4	++++	4	4	++++	4	6	+	7	10	++	4	5
D	++++	6	7	++++	4	5	++	3	6	++++	3	3	+	4	6	++	4	4
E	++++	6	7	+++⊥	4	3	++++	4	4	+++	4	6	++++	6	7	+++	4	5
G	++++	5	8	++++	4	3	+++	4	4	+++⊥	4	6	+++⊥	7	10	++++	4	3
Totals		36	45		24	23		23	26		21	30		38	53		24	27

Total chronic cases 166
Total control cases 53*
Key to abbreviations:

− no immunity
+ low grade partial immunity
++ medium grade partial immunity
+++ high grade partial immunity
+++⊥ very high grade immunity
++++ maximum immunity

* The number of controls is less than would appear from other figures on the table because several controls, in many cases, served for an entire series consisting of chronic cases of a number of strains.

drawn from them. In a later paper, Et. Sergent and Catanei (1937) found that serum from birds infected with *P. relictum* produced no preventive or immunizing effect. They inoculated very small doses of malarial serum subcutaneously before, during, and after new infections had been produced in experimental birds.

From these first attempts to demonstrate humeral antibodies in bird malaria infections it was assumed that no such protective mechanism existed, but recently several papers have appeared which indicate that the problem is by no means solved. A procedure common to all of the papers mentioned above is that comparatively small doses of serum were used. Coggeshall and Kumm (1937), however, found that if large doses of malarial serum are used protectively in monkeys infected with *P. knowlesi* the severity of the attack is decreased, and consequently humeral antibodies are probably present. Hegner and Eskridge (1938c) and Hegner and Dobler (1939) were similarly able to demonstrate a certain amount of protective action in *P. cathemerium* infections in canaries when large doses of serum from birds suffering from active or subpatent infections were injected into them. In the first of these papers, dried, pulverized serum from uninfected and infected birds was obtained. This was dissolved in saline and was used to treat experimental birds previous to their inoculation with viable parasites. In three birds treated with large doses of acute serum no symptoms and little pathology was exhibited, in contrast to control birds and those treated with latent serum. The parasite number, however, in the birds treated with acute serum was not noticeably less than in the other birds (figure 24). The authors therefore conclude that the acute serum protected against the toxic products resulting from the infection, without decreasing the parasite number. In repeating the above experiments, using acute serum heated to 56° C. for 30 minutes, Hegner and Dobler (1939) found that protection was not conferred in experimental birds against either the toxic substances or the number of parasites. Dried, acute serum, however, appeared to protect to a slight extent as evi-

denced by a decrease in the severity of the pathology exhibited in the spleen and liver. Similarly, injections with fresh and dried spleen gave only slight protection.

More pronounced results are presented by Manwell and Goldstein (1938c) in *P. circumflexum* infections. Large amounts of serum from infected birds were injected into unparasitized canaries, and the latter were completely protected against subsequent inoculations with viable parasites. Less effective protection was given when the serum was injected into birds already parasitized. The degree of immunity was greater in the birds inoculated with parasites of the same strain used in the birds from which donor serum was obtained. W. H. and L. G. Taliaferro (1939) report that serum from chickens immunized with *P. lophurae* exerts a protective action against infection in non-immune chickens which varies with the size and frequency of the serum treatments and the number of parasites given the test animals.

In summarizing all of the results thus far obtained it seems apparent that humeral antibodies of some kind do exist in the blood serum of birds infected with some strains of avian plasmodia. That they occur in small concentration is evidenced by the failure of early workers to demonstrate their presence when small doses of immune serum were used, and the success of later workers who tried larger amounts. The nature of these protective substances has not yet been demonstrated, but their action probably supplements the cellular response in ridding the host of parasites. In human malaria, Sotiriadés (1936), and Lorando and Sotiriadés (1936) have obtained favorable results by treating children with immune maternal blood.

5. RELAPSE

The phenomenon of relapse in human malaria has been described by James as " a return of the fever and parasites, after the former has ceased and the latter have disappeared from the peripheral blood, reinfection being excluded." An exactly comparable occurrence takes place in birds, and the recurrence of parasites in the blood after recovery from the

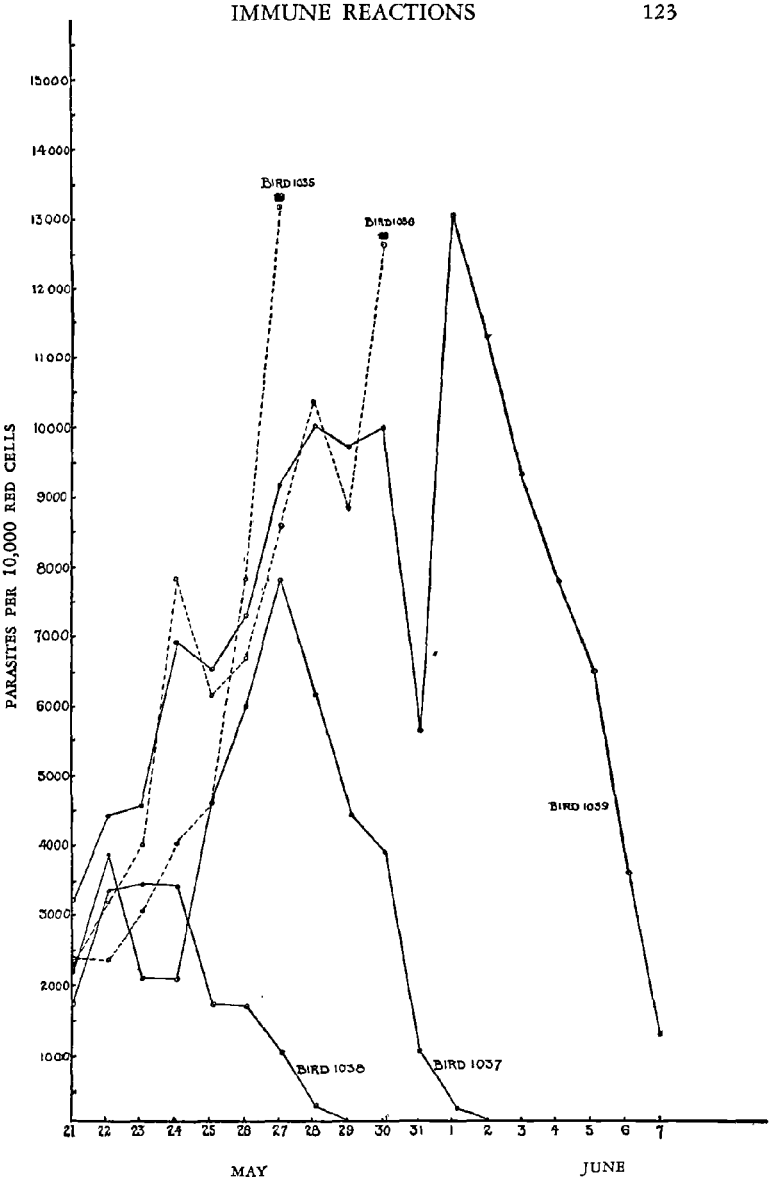


FIGURE 24—Curves of parasite numbers in two canaries (1035, 1036) treated with serum from subpatent birds, and in three canaries (1037, 1038, 1039) treated with serum from birds suffering with acute infections (from Hegner and Eskridge, 1938).

primary attack is characteristic of certain species and strains of avian plasmodia. The factors which determine relapse undoubtedly undermine the host defense mechanism in some way, and the subject is therefore properly included in the discussion of immune responses.

Danilewsky (1889) followed infections in wild birds for long periods of time, and some of the chronic periods which he observed were probably the result of frequent relapses. Wasielewski (1901) likewise noted recurrent increases in the number of parasites in the blood of some of his birds, and Moldovan (1912) made direct efforts to produce relapse experimentally by injecting parasitized and unparasitized blood from rice birds into his experimental hosts. Some relapses actually occurred after this treatment, even though non-parasitized blood was used as a provocative. Whitmore (1918) in a brief set of experiments followed Moldovan's clue and concluded that relapse may occur as a result of lowered resistance from intercurrent infections, or of the injection of foreign blood. Whitmore (1922) was also able to produce relapses in birds carrying latent infections with *P. relictum* by exposing them for 2 hours to the unfiltered light of a quartz mercury vapor lamp at a distance of 24 inches. When the feathers were removed from the bird, an exposure of 15 minutes was sufficient to produce relapse.

The first real attempt to discover some of the principles underlying the mechanism of relapse was undertaken by Ben-Harel (1923). She observed that some birds relapsed at varying intervals in spite of the fact that the surrounding conditions had not changed in any way. Experimental relapse was produced in some cases by radiation with ultraviolet rays or by the injection of adrenalin. Studies on the bone marrow and spleen in latent cases led her to believe that these organs remained as sites of infection long after the parasites had disappeared in demonstrable numbers from the peripheral blood stream. All stages of the normal asexual cycle were found in these organs, indicating that the cycle proceeded as usual during latency, although only small numbers of asexual forms survived. Any influence which

might cause a decrease in the usual destructive mechanism operative in keeping the parasites down to a low level during latency could therefore result in an increase in the number of parasites already present in small numbers throughout the body. L. G. Taliaferro (1925) observed that parasites reproduce at the same rate as long as they are found in the blood, and likewise when they reappear during a relapse they still reproduce at the same rate. The various parts of

TABLE 16

MONTHLY VARIATION IN RELAPSE RATE (*Plasmodium cathemerium*) (FROM MANWELL, 1929)

Month	Total cases	Total relapses	Relapse percentage
January 1928	90	12	13.3
February 1928	96	6	6.2
March 1928	95	7	7.4
April 1927	50	1	2.0
May 1927	56	3	5.4
June 1927	58	4	7.0
July 1927	67	0	0.0
August 1927	60	3	5.0
September 1927	63	7	11.1
October 1927	65	11	16.9
November 1927	70	7	10.0
December 1927	82	15	18.3

Note: No relapses occurring sooner than six weeks after the date of inoculation are included in this table.

the cycle also occurred at the same hour of the day after parasites had recurred in the blood stream. This denotes that during latency the same asexual cycle persists, even though the parasites are greatly reduced in number, so that when a circumstance occurs which unbalances the host-defense mechanism the parasites are ready and able to continue their life cycle on a larger scale in exactly the same way as during the primary attack.

Manwell (1929) furthered our knowledge of relapse phenomena as a result of extensive observations involving 332

infections with *P. relictum, P. cathemerium* and *P. elongatum.* Both short-term and long-term relapses occurred, and the former appeared to be the most common. Seasonal variations were noted in the frequency of relapse in infections with *P. cathemerium* (table 16). When the environmental conditions were carefully controlled the greatest number of relapses was noted in Fall and Winter, and the smallest number in Summer. Differences also occurred in differ-

TABLE 17

VARIABLE SUSCEPTIBILITY OF BIRDS TO RELAPSE
(FROM MANWELL, 1929)

Number of cases	Number of relapses	Percentage of total birds
21	0	60.0
5	1	14.3
4	2	11.0
2	3	5.7
1	4	3.0
1	8	3.0
1	11	3.0
35	42	100.0

ent species; the W strain of *P. relictum* produced relapses in 61 per cent of the cases, and *P. cathemerium* in only 5.5 per cent. Some birds relapsed at very short intervals continuously, as many as 8 or 10 times (table 17). Relapses occurred more frequently immediately following the primary infection, and less frequently 3 months after inoculation. Quinine and plasmochin treatment during the acute stage of the infection seemed not to exert influence on the subsequent frequency of relapse. No relapses occurred when latent birds were exposed to direct sunlight for 2 days.

Et. Sergent (1934) has reported that relapses in malaria-infected birds occur more frequently during the period between the first quarter of the moon and the full moon, and when the maximum barometric pressure is low.

All of these points give useful information as to the conditions which produce relapse, but no one has as yet demonstrated the fundamental mechanism which brings about the decreased resistance on the part of the host, thus causing relapse. Several conditions known to produce relapse (ultraviolet light, X-rays, low barometic pressure, seasonal variation, etc.) are associated with an increase in young red cells (Hegner, 1938), and it is known from the researches of Hegner and Hewitt (1937, 1938), and Hegner and Eskridge (1938a) that the merozoites of some species of bird malaria parasites penetrate young red cells. Whether or not these are coincidences has not yet been fully demonstrated, but further investigations are indicated along this line.

6. HOST-PARASITE SPECIFICITY

The different species of avian plasmodia differ considerably in their degree of host-parasite specificity. Both loose and rigid host-species immunity occurs. *P. relictum* and *P. cathemerium*, for example, have been found in nature in many species of wild birds, and they are always readily inoculable to the canary. Wolfson (1938c) has recently been successful in inoculating strains of *P. relictum* and *P. cathemerium* to ducks, although the infections produced in this type of host are not so severe as in canaries. These two species might then be considered as exhibiting loose host-parasite specificity, since their occurrence in widely different bird hosts is common. *P. vaughani* and *P. polare* on the other hand seems to exhibit a rigid host-parasite specificity, since to date the former has been found in nature only in robins, and the latter in cliff swallows, although both are inoculable to canaries (Manwell, 1938b). This is not to be regarded too seriously, however, since it is doubtful whether either of the two species mentioned can be diagnosed accurately in surveys of wild birds unless the investigator is familiar with them. Future surveys may reveal that even these species are less specific with regard to their hosts than is now believed.

Various intermediate degrees of host-parasite specificity

exist in the other species of bird malaria. *P. gallinaceum* can be inoculated successfully to geese, but not to canaries or ducks (Brumpt, 1936b). *P. circumflexum,* although not so cosmopolitan in distribution as *P. relictum*, seems to occur commonly in various passerine birds (Manwell and Herman, 1935b). The immune mechanisms of each type of host in repelling certain types of parasites and not others are not at all well understood in any type of parasitism, and practically no information is as yet available which throws light on the problem in bird malaria.

7. OTHER ASPECTS OF IMMUNITY

a. *Henry's reaction.* Kritschewski and Demidowa (1933) and Brumpt and Chorine (1937) have investigated melanoflocculation in birds infected with malaria. The former authors used *P. relictum* and *P. cathemerium,* and concluded that antibodies are formed in infected birds against the malaria pigment, and that these react with melanin to form a flocculent precipitate in serological tubes. Brumpt and Chorine, on the other hand, found that the serum of normal fowls gave a positive Henry's reaction, and that the abundance of malaria pigment in birds infected with *P. gallinaceum* did not increase the intensity of the flocculation. It seemed clear to these authors that the malaria parasite was in no way responsible for the reaction.

In view of the conflicting opinions which exist concerning melanoflocculation tests in human malaria, it seems unwise to base conclusions on its efficacy in avian infections from the little data available. Whether or not it demonstrates an anti-body response is questionable at present.

b. *The electric charge of parasitized erythrocytes.* The work of Brown (1933) and Findlay and Brown (1934) indicates that the serum of infected birds undergoes some change which results in a reduction in the charge of parasitized cells. Brown makes the following statement: "The electric charge of the erythrocytes in birds infected with bird malaria bears an inverse relation to the parasite rate. It ap-

pears that the reduction of charge in the red cells of an infected bird is an important factor in the extent to which phagocytosis takes place." Whether or not this difference in charge is of sufficient importance to be included as one of the factors governing immunity will not be definitely known until the work is repeated.

c. *The effect of splenectomy on the course of infections.* Although considerable evidence exists to the effect that splenectomy in apes and monkeys infected with malaria results in relapses, little work has been done on this subject in bird malaria. Brumpt (1936b) reports relapses following splenectomy in two birds suffering from *P. gallinaceum* infections. Low grade and short-lived infections with *P. circumflexum* were obtained in splenectomized chicks by Herman and Goldfarb (1939), but the results were not conclusive enough to demonstrate that the removal of the spleen noticeably reduces resistance. Causey's (1939) results in *P. relictum* and *P. cathemerium* infections (in canaries) following splenectomy indicate that the absence of the spleen does not materially alter the course of infections. Relapses resulted, however, when the spleen was removed from birds with latent malaria, and death occurred in 2 out of 3 such cases.

d. *Vaccination.* It has already been mentioned that the mechanism of immunity in bird malaria infections seems to depend upon the presence of living organisms within the body of the host, and that protection is lost when complete natural recovery occurs. This raises the question as to whether killed or attenuated plasmodial protoplasm will elicit the formation of antibodies when injected into a non-immune host. The Sergent brothers and Catanei have made several attempts to demonstrate vaccination against bird malaria parasites with variable results. In 1921 (a) Edm. and Et. Sergent tried the following procedures: (1) modifying the site of inoculation (e. g. rectal inoculation), (2) injecting serum from animals which had received doses of *P. relictum* but which were naturally immune to this parasite (pigeons, guinea pigs, etc.), (3) modifying the infective

blood used for inoculation (low temperature, treatment with chemicals), (4) modifying sporozoites in a similar fashion before inoculation, and (5) injecting parasites not yet mature (zygotes in mosquitoes, blood of infected canaries during the prepatent period). They achieved positive results in 29.5 per cent of 24 cases in which "old" sporozoites, conserved *in vitro* for 12-48 hours, were inoculated into canaries before the introduction of viable parasites, and in 21.3 per cent of the cases in which peripheral blood parasites taken during the prepatent period were inoculated into birds before viable parasites were introduced. In a subsequent paper the Sergents and Catanei (1923a) report successful vaccination by using sporozoites in varying dosages. Large doses of sporozoites produced high infections in birds. Smaller doses produced lighter infections with resulting immunity to superinfection in most cases. The optimum dosage of sporozoites for the most successful protection was found to be ⅔ or ¾ the unit body of infected mosquitoes, the unit body being the number of sporozoites contained in a single mosquito naturally infected. No immunity or feeble immunity was obtained if less than ⅔ the unit body was used. In 1934 the same authors found that blood heavily parasitized with *P. relictum*, when mixed with citrate solution and glycerin and kept at room temperature for 4 days, did not produce infections when inoculated into parasite-free canaries, and furthermore did not immunize the birds against subsequent inoculation with viable parasites.

A more recent report by Redmond (1939b) describes successful vaccination by attenuating parasites at -3° to -4° C. for 73-96 hours and using this material as the vaccine. Intravenous injections of the treated parasites were given every other day until 8 or more injections had been given, and viable parasites were then inoculated into the test birds, along with appropriate controls. The control birds showed typical infections. Seven of the test birds were completely immune, and three test birds were partially immune. The author therefore concludes that the vaccination was successful. It is well known, however, that freezing or sub-freezing

temperatures do little more than inhibit the growth of malaria parasites. When inoculated into parasite-free birds refrigerated parasitized blood will produce an infection, although the patent period is generally prolonged. These results, then, hardly demonstrate true vaccination, but seem rather to add additional evidence that living parasites must be present in the host in order to produce immunity against superinfection.

CHAPTER VIII

THE EFFECTS OF DRUGS AND CHEMICALS ON INFECTIONS

A. CHEMOTHERAPY

The similarity of avian and human malaria is more striking in their response to anti-malarial drugs than in any other respect; this fact has stimulated the search for new curative chemicals. No drug yet found completely eliminates all of the parasites from either the human or avian host in all cases of infection, and many gaps still remain to be filled regarding our knowledge of the action of drugs which are known to produce beneficial effects. It is not surprising, therefore, that the useful laboratory tool available in the plasmodia of birds has served as the testing device for a multitude of trials in the hope of perfecting a true malaria-specific chemical. Although quinine was known to alleviate the suffering in human malaria long before the time of Danilewsky it was not until bird malaria parasites were discovered that an experimental host became available upon which to test the effects of other chemical compounds. As pointed out by Kikuth and Schönhöfer (1935), without the method of administering drugs to canaries infected with bird malaria it would scarcely have been possible to discover plasmochin and atebrin so quickly.

In presenting the brief resumé which follows of some of the results which have been achieved in bird malaria therapy, all of the experimental work cannot be given the credit which it deserves, due to the limitation of space. Representative papers are quoted, illustrative of the particular points discussed, and a full bibliography is given at the end of the monograph. References to many publications are omitted, particularly when the results therein given are not essentially different from the work which is quoted.

1. HISTORICAL

The effect of quinine on avian plasmodia was first observed by Wasielewski in 1904. He noted that the parasites were killed *in vitro* by a concentration as low as 1: 10,000 at room temperature. Kopenharis (1911) confirmed this work, although the concentration of quinine necessary to kill the parasites *in vitro* was said to be 1:1200, and a dilution of 1: 2000 did not kill all of the plasmodia. The Sergents (1921c) conducted the first extensive series of experiments on the effect of quinine *in vivo* in bird malaria infections. They showed that the drug in the doses which they employed was effective in holding the parasites in check for as long as treatment was continued, although relapse frequently occurred afterward. Similar results, varying only in degree, were obtained by the Sergents and Catanei (1924b), Giemsa, Weise and Tropp (1926), G. H. Boyd (1926), Kikuth and Tropp (1927), Katahira (1926), and others.

The work of Roehl (1926), followed by that of Hegner and Manwell (1927), introduced the synthetic compound plasmochin, and the effect of this drug on avian plasmodia resulted in its adoption in treating human malaria. With the exception of the last paper mentioned all of the above work seems to have been carried out with *P. relictum* infections. Hegner and Manwell (1927) used *P. cathemerium* in their work.

The discovery of plasmochin greatly stimulated research on chemotherapy, and during the years 1927 to 1935, the number of papers published annually on the effect of various drugs rapidly increased. Most of these are listed in the bibliography, but several command particular attention. Hegner, Shaw and Manwell (1928), and Shaw (1928) tested the effect of many compounds which had not been previously tried (*P. cathemerium* infections), including members of the quinine, quinolene, substituted phenol, azo dye, pyridine, and organic metallic series. Fifty-eight different chemicals were tried, but it was found that members of the quinine series produced the best results. Manwell (1930a and b, 1934a) furthered these experiments and was the first to compare the

effects of quinine and plasmochin on different species of bird malaria (table 18). Kikuth's success (1932a) with the new drug atebrin again provided an impetus for further investigations, and the list of compounds used for therapeusis steadily increased thereafter. Among these may be mentioned " 710 Fourneau " (Sergents *et al,* 1931), l'ichthargon (Sergent and Catanei, 1932a), totaquine and hydroquinone (Giemsa, 1933), " R 123 " (Sternberg, 1934), methylene blue (Albricht and Nieuwenheyse, 1937), " Paludex " (Rodhain and Hendrix, 1937), and " Certuna " (Kikuth, 1938a).

Thus far the goal of these investigations has not been reached, in that no drug or chemical has been found which eliminates all of the parasites from the body of the host, although this has been accomplished by Coggeshall (1938a) with sulphanilamide in monkeys infected with *P. knowlesi.* Some cases of sterilization have been reported in infections with some species of bird malaria, but these are by no means constant. As new chemicals and compounds are devised for the treatment of many human ills other than malaria, they usually find their way into the peripheral blood stream of malaria-infected canaries in the hope that one of them will prove to be a true malaria specific.

2. DOSAGE AND METHODS OF ADMINISTRATION

As mentioned in a preceding chapter, drugs or chemicals may be introduced into birds orally, intramuscularly, intravenously, or intraperitoneally. The esophageal tube was first used by G. H. Boyd (1926) and was popularized by Roehl (1926) for administering drugs orally. The dosages used by different investigators vary considerably and a standard procedure cannot be given which will cover all types of experiments. The following sample doses (Manwell, 1930a, 1933c) may be taken as representative of the general procedure followed:

(a). Quinine. 0.75 mgms. are dissolved in 100 mgms. of distilled water. Canaries will tolerate an oral dose of this size daily for 8 weeks without apparent injury.

TABLE 18

THE RELATIVE EFFECTS OF QUININE AND PLASMOCHIN THERAPY UPON FIVE SPECIES OF BIRD MALARIA †
(FROM MANWELL, 1934)

	PLASMODIUM CATHEMERIUM		PLASMODIUM CIRCUMFLEXUM		PLASMODIUM PRAECOX		PLASMODIUM ELONGATUM		PLASMODIUM ROUXI	
	Plasmochin	Quinine	Plasmochin	Quinine	Plasmochin	Quinine	Plasmochin	Quinine	Plasmochin	Quinine
Days required to reduce parasite-level below point of visibility.	2.17± 0.47	3.66± 2.23	1.80± 0.59	2.77± 0.62	3.00± 0.38	5.00± 0.48	2.25± 0.45	7.50± 3.16	2.29± 0.30	3.43± 0.79
Percentage of parasite-free days.	67.85± 11.03	59.50± 21.11	86.03± 4.55	57.94± 8.61	76.91± 3.33	62.01± 4.70	84.03± 3.17	33.20± 16.62	83.67± 2.18	75.51± 5.68
Total cases sterilized ‡.....	0	0.	4(5?)	0	7	1	14	1	18	12
Total cases treated.....	12	12	18	19	26	27	14	12	18	14
Percentage sterilized.....	00.0	00.0	22.2 (27.7)	00.0	26.9	3.7	100.0	8.3	100	85.7

† All cases included in this table were treated with the same dosage and for the same length of time (two weeks).

‡ By "sterilized" is meant not only the cases which actually showed parasites before treatment began and were then completely cured, but also those in which the infection was completely aborted by treatment during the incubation period.

(b). Plasmochin. 0.132 mgms. are dissolved in 100 mgms. of distilled water. The dose is given orally in two parts daily for 8 weeks without apparent toxic effect to the host.

(c). Atebrin. 2 mgms. are dissolved in 100 mgms. of distilled water. This is given in two doses each day, orally, for from 7 to 9 days.

In all of the above cases 100 mgms. is used as a standard dose for a canary weighing 16.5 grams. Six mgms. of solution is added or substracted from each gram variation from this figure.

TABLE 19

SHOWING THE MORTALITY ASSOCIATED WITH DAILY TREATMENT BY SINGLE INTRAPERITONEAL INJECTIONS OF QUININE HYDROCHLORIDE IN VARIOUSLY-SIZED DOSES (FROM LOURIE, 1934)

Size of daily dose per 16.5 gm. bird	Number of birds treated	Died on 1st day of treatment	Died during 1st week of treatment	Died during 2nd week of treatment	Survived 2 weeks' treatment	Percentage of survivors after 2 weeks' treatment
4 mgms.	7	3	4	..	0	0
3 mgms.	7	0	6	1	0	0
2 mgms.	32	0	9	13	10	31.2
1 mgm.	221	0	11	27	183	82.8

Table 19, taken from Lourie (1934c), shows the mortality in canaries associated with daily injections of variously-sized doses of quinine administered intraperitoneally. The daily intraperitoneal injection of 1 or 2 mgms. of quinine hydrochloride in 0.1 cc. of distilled water did not cause severe local reactions in the majority of cases, and 82.8 per cent of the birds treated daily with 1 mgm. of quinine survived two weeks treatment.

In testing the effects of drugs for which the minimum lethal dose is not known, this must be determined, and must be done by the trial and error method if the toxic effect of the agent employed cannot be approximated by any other means.

3. METHODS OF SELECTING DRUGS

It has been assumed that to be effective against malaria a compound must penetrate the red blood cells. Hegner, Shaw and Manwell (1928) tested the *partition coefficient* of the drugs they used (e. g. the concentration of the drug in the corpuscles divided by the concentration of the drug in saline solution when mixed with the corpuscle suspension). Drugs with a high partition coefficient are absorbed in greater degree by the red cells than by the serum. Shaw (1928) found that only those compounds which do not have ionic groups are absorbed.

Another method of selection is on the basis of lethal strength for free-living protozoa (e. g. *Paramecium*). This is also described by Hegner, Shaw and Manwell (1928). The toxicity of drugs to be tested was determined by finding the degree of toxicity to *Paramecium caudatum.* The fact that quinine and related alkaloids are toxic to *Paramecium* in proportion to their effectiveness in the treatment of malaria indicates that this method may be of some real value.

Synthetic derivatives similar to compounds of known malaricidal action frequently, but not always, exhibit similar properties. Long chains of compounds may thus be tried when one member of the group is found to be effective against plasmodia.

The behavior of chemicals *in vitro* with malaria plasmodia has not been successful, since a satisfactory method of cultivating bird malaria parasites has not yet been devised.

4. THE MODE OF ACTION OF DRUGS EFFECTIVE AGAINST PLASMODIA

Wasielewski (1904), Kopenharis (1911), and Lourie (1934c) have observed the action of quinine when placed in a suspension of infected corpuscles. The last author found that *P. cathemerium* will resist incubation at 39° C. with quinine (1:500) for 1 hour, since the parasites are infective after this treatment. The concentration necessary to delay segmentation is about 1:5000, with exposures of from 1 to

2 hours. The author concludes that this is suggestive that the action of quinine *in vivo* is not a direct one on the part of the unaltered drug, since the very strong concentration mentioned could not be maintained in the blood stream for any length of time.

Wampler (1930) reports degenerative changes in the trophozoites and segmenters of *P. cathemerium* after quinine treatment, and Manwell and Haring (1938) find similar changes in *P. vaughani* infections treated with atebrin.

The differences in the response of various species of avian plasmodia to quinine and plasmochin is thought by Manwell

TABLE 20

LIST OF SPECIES OF AVIAN PLASMODIA IN THE ORDER OF THEIR SUSCEPTIBILITY TO QUININE AND PLASMOCHIN

(FROM MANWELL, 1934)

Quinine	Plasmochin
1. *Plasmodium rouxi*	1. *Plasmodium elongatum*
2. *Plasmodium relictum*	*Plasmodium rouxi*
3. *Plasmodium cathemerium*	2. *Plasmodium relictum*
4. *Plasmodium circumflexum*	3. *Plasmodium circumflexum*
5. *Plasmodium elongatum*	4. *Plasmodium cathemerium*

(1934) to be correlated with differences in their reproductive rates. Table 20 lists 5 species in the order of their susceptibility to quinine and plasmochin. Lourie (1934d) observed a considerable difference between strains of *P. relictum* and their response to quinine treatment, but offers no explanation for this.

A distinct retardation of schizogony in *P. cathemerium* infections treated with quinine was noted by G. H. Boyd and Allen (1934), and G. H. Boyd (1933) states that a decline occurs in the number of merozoites produced by each segmenter following quinine treatment. This is confirmed by Lourie (1934c); the infections which he treated with quinine exhibited retardation of growth, delay in segmentation, and

a reduction in the number of merozoites produced (figure 25). Synchronicity was completely abandoned. G. H. Boyd and Dunn (1939) found similar changes following both quinine and plasmochin treatment.

These results indicate that the effect of malaricidal drugs is not confined to a single action, but rather that a number of morphological and physiological changes in the parasite take place in the presence of the chemicals, and that the combined action of these inhibiting effects reduces the parasite number. It has not been definitely shown how the chemicals attack the parasites, but the effects of the attack are quite clearly indicated in the papers which have appeared on the subject.

5. USE OF MALARICIDAL DRUGS IN RESEARCH NOT DIRECTLY CONCERNED WITH THERAPY

Brumpt and Bovet (1936) found that Fourneau 852 acts on *P. relictum* but not on *P. paddae,* although the two species are morphologically indistinguishable. In the light of Manwell's work on the response of different species to quinine and plasmochin this work is further substantiated. Lourie (1934d), however, observed marked differences in the effect of quinine on different strains of *P. relictum* so that the procedure recommended by Brumpt and Bovet is not indubitable proof that *P. paddae* is not synonomous with *P. relictum.*

Hegner and Eskridge (1938c) believe that if humeral antibodies are present in the serum of heavily parasitized birds these antibodies will be available in higher titer if the birds are saved from death by administering quinine. A similar idea was used by Coggeshall and Kumm (1937) in infections with P. *knowlesi* and *P. inui* in rhesus monkeys. Both of these papers present evidence to show that the severity of the malarial attack is reduced when serum from quinine-treated animals is injected into animals suffering from bird or monkey malaria.

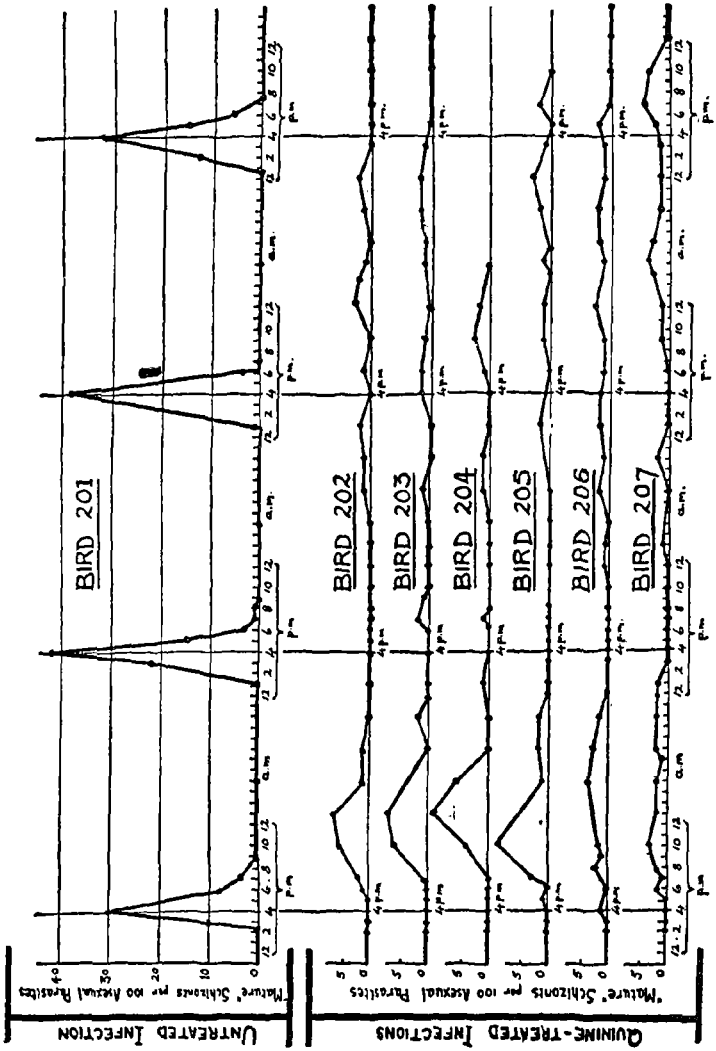


FIGURE 25—Graph illustrating the marked influence of daily quinine treatment of the host upon reproduction in *P. cathemerium* (from Lourie, 1934).

B. The Action of Chemicals Other Than Those Used For Therapy

1. GLUCOSE

In studies on the effect of changing the sugar content of the blood in bird malaria, Hegner and MacDougall (1926) and MacDougall (1927) demonstrated that increased sugar in the blood modifies the course of the infection, bringing about conditions favorable for the parasites and hence prolonging the infection (*P. cathemerium*) until death results. Figure 26 illustrates the type of infection encountered after feeding the bird dextrose, as compared with a control infection. This is in agreement with the work of Bass and Johns, and others, in which glucose was shown to be necessary for the successful cultivation of human plasmodia *in vitro.*

2. INSULIN

Conversely to the effect of glucose, MacDougall (1927) found that the administration of insulin throughout the course of infections with *P. cathemerium* reduced the number of parasites in the peripheral blood, as compared to control birds. This is illustrated in figure 27. The effect of insulin is presumably that a reduction in the amount of blood sugar takes place following its administration, making the blood stream unfavorable for the development of parasites. It is in no sense a cure for malaria, however, since the effect is relative and does not sterilize the infected bird. When insulin was given to infected birds for 6 days, discontinued, and followed by glucose, the number of parasites increased, as shown in figure 27.

3. PHENYLHYDRAZINE HYDROCHLORIDE

Hegner and Hewitt (1938) and Hewitt (1939c) gave phenylhydrazine hydrochloride to canaries before inoculating them with *P. cathemerium,* in order to increase the number of young red cells in the peripheral blood (phenylhydrazine

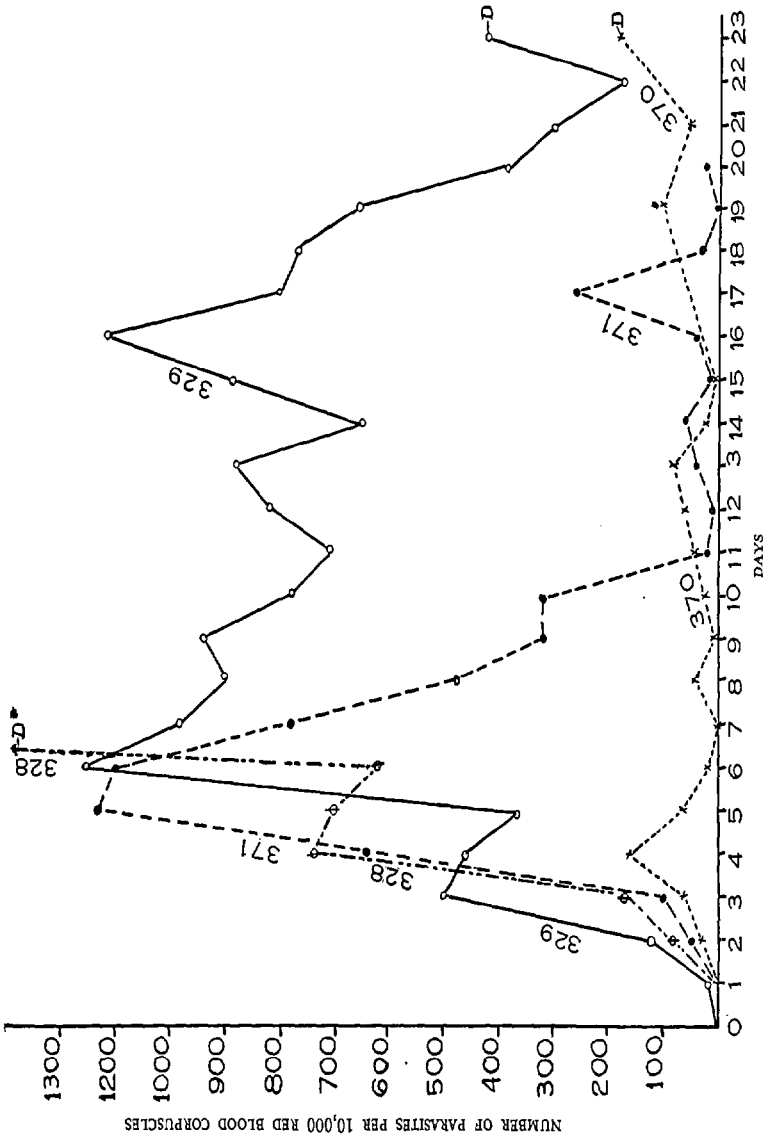


FIGURE 26—Curves showing the effect of dextrose fed throughout infections with *P. cathemerium*. D, signifies the death of the bird (from MacDougall, 1927).

destroys red cells while it is present in the blood and thus stimulates haemopoeisis). The number of parasites in phenylhydrazine-treated birds reached a higher peak than in untreated controls (figure 5). Parasite counts in treated birds were from four to six times greater per cubic millimeter of blood at the peak of infections than in controls. The only difference known to exist between phenylhydrazine-treated birds and controls is the difference in the number of young red cells before parasites are inoculated, more of these occurring in the treated birds. Since merozoites penetrate young red cells in normal infections with *P. cathemerium* it is concluded that the presence of large numbers of young cells in the phenylhydrazine-treated birds favors the survival of more parasites than in controls. No evidence was obtained which indicated that the chemical produced a toxic effect on the host other than stimulating haemopoeisis through the destruction of red cells.

4. COLCHICHINE

This chemical has been shown by physiologists to arrest cell-division at metaphase by inhibiting the formation of an achromatic spindle. Coatney and Young (1939) tested its effect on *P. relictum* in pigeons in an attempt to determine whether it would produce a growth inhibiting effect on segmentation. Under the conditions of the experiments performed no retarding effect upon the prepatent period or upon the division of *P. relictum* occurred. The authors conclude that if an achromatic figure exists in bird malaria it is not affected by this chemical. On the other hand, the non-effectiveness of colchichine may suggest the absence of such a division figure in bird malaria parasites.

CHAPTER IX

THE SEXUAL CYCLE AND MOSQUITO TRANSMISSION

A. DESCRIPTION OF SEXUAL STAGES

1. SEXUAL STAGES IN THE VERTEBRATE HOST

From the very first work on bird malaria the large oval or elongate gametocytes filling most of the red cell were recognized and illustrated, although their true significance was not appreciated until MacCallum (1897) demonstrated that they represented sexual stages of the parasite. In much of the early literature the gametocytes of *Haemoproteus* were undoubtedly confused with those of *Plasmodium*, but the species with elongate or halter-shaped gametocytes were clearly separated from those exhibiting round gametocytes (Grassi and Feletti, 1891). Opie (1898) described the appearance of both types of gametocytes in unstained as well as in stained preparations, but did not realize the importance of the distinction at that time. MacCallum, however, observed granular (macrogametocytes) and hyaline (microgametocytes) forms escape from red blood corpuscles in drawn blood taken from a *Haemoproteus* infection, and discovered the true nature of these bodies when exflagellation and fertilization occurred. Most subsequent investigators were able to recognize and distinguish between the two types of gametocytes which are found in the peripheral blood of birds infected with plasmodia.

In unstained preparations of drawn blood in saline the macrogametocyte appears more granular and contains greater amounts of pigment than does the microgametocyte, which is more or less clear or hyaline in appearance. When stained by one of the Romanowsky methods the female gametocyte appears to be a deeper blue than the male, and the nucleus is more dense and compact. Gambrell (1937) states that a

conspicuous karyosome is visible in the chromatin substance of the female gametocyte of certain strains of *P. cathemerium* and *P. relictum*, while such a structure is not always present in the male gametocyte. The presence of a karyosome in both male and female gametocytes has been confirmed in this laboratory.

The shape of both types of gametocytes is one of the distinguishing characteristics used to differentiate species (plate V). *P. relictum, P. cathemerium, P. gallinaceum* produce round or oval gametocytes, which displace the nucleus and generally lie in one end of the host cell. Species of the *P. circumflexum* type, including *P. lophurae, P. heroni,* and others, exhibit halter-shaped gametocytes which encircle the nucleus without displacing it, and are thus similar to most species of *Haemoproteus.* It is difficult to differentiate these species from *Haemoproteus* if segmenting forms are not present in the peripheral blood. The gametocytes of *P. rouxi, P. elongatum* and most of the smaller species of avian plasmodia are generally narrow and elongate, but seldom curve around the polar ends of the nucleus to any great extent. Illustrations of these various shapes are presented in plate V.

It is generally accepted that gametocytes originate from asexual forms, and that certain of the merozoites produced by segmenters are destined to become sexual cells. The mechanism of gametocyte production is not well understood, but the genetic constituents of the nuclei probably determine whether a parasite will become an asexual or a sexual form. Shah (1934) seems to have been the first worker to consider seriously the development of gametocytes from young trophozoites. In preparations of *P. cathemerium* he claims he was able to distinguish young sexual forms from young asexual parasites within about 8 to 10 hours after segmentation. He believes that trophozoites which are elongate in shape and contain pronounced rod-like pigment granules eventually become gametocytes. The differentiation at this early stage is not marked, and to the inexperienced is extremely difficult. At the end of about 14 hours following segmentation most of the asexual parasites have started to divide and at this time the gametocytes can be more definitely

separated from them, since the nucleus of the sexual forms does not undergo division.

Gambrell (1937) believes that the presence of a karyosome in the premacrogametocyte is a dependable characteristic for differentiating immature sexual forms from asexual forms, although this structure may be lacking in the premicrogametocytes.

2. THE RELATION OF GAMETOGENESIS TO SCHIZOGONY AND THE PATENT PERIOD

In infections with *P. cathemerium*, L. G. Taliaferro (1925) observed that the percentages of gametocytes remained at about 3 per cent of the total parasite count throughout the acute period, but increased from 2 to 50 per cent during relapse and sometimes during the chronic period. Huff (1927) found that the percentage of gametocytes in infections with the same species rose continuously from the time of their appearance, and that the maximum number occurred one or two days after the maximum total parasite number. Shah (1934) and Gambrell (1937) state that gametocytes of *P. cathemerium* appear simultaneously with the asexual forms and increase in numbers as the asexual forms increase (figures 11 and 12). They decrease in number when the total parasite number drops.

The growth of gametocytes from young, undifferentiated trophozoites to mature sexual forms was observed by Shah (1934) to occur within approximately the same period of time as that required for the complete maturation of asexual parasites, namely 24 hours (*P. cathemerium*). Furthermore, this growth to maturity seemed to take place in the peripheral blood and was not dependent upon intervening stages in the visceral organs. This was confirmed by Gambrell (1937). She found that pregametocytes are more prevalent at the segmentation time of asexual parasites, the numbers are augmented periodically, and new broods are distinguishable, although the time for maturation was said to be more than 24 hours, usually 30 to 36 hours. The methods used by these investigators for determining the periodicity of

sexual forms were the same as those commonly practiced in periodicity work on the asexual stages.

3. GAMETOCYTELESS STRAINS

Huff (1934), Huff and Gambrell (1934), and Gambrell (1937) have described at least two gametocyteless strains of *P. cathemerium,* and these are as yet the only strains of bird malaria which have been found to lack sexual stages in their life cycle. The " F " strain, first described by Huff, and Huff and Gambrell, was discovered in the course of a series of subinoculations of the " D " strain of *P. cathemerium,* and the " N " strain originated from a deviation of a single-cell strain of *P. cathemerium* isolated by Stauber. In the latter case the gametocyteless strain appeared in a bird in which the course of infection had been altered by light and dark experimentation.

One of the outstanding characteristics of the gametocyteless strains is the lack of synchronism in the asexual reproductive cycle (figure 28). Morphological differences are also noticeable. The parasites are not distinct in their outlines, stain slightly paler than the typical *P. cathemerium,* and are described as being " ragged " in appearance. The number of merozoites in mature segmenters in the " F " gametocyteless strain is 4.95 $\pm$ .18 less than in the typical strain.

Complete cross-immunity has been shown to exist between the " F " gametocyteless strain and its parent strain, although the gametocytes of the superimposed strain were not removed as readily as the asexual forms.

Attempts to infect susceptible mosquitoes with gametocyteless strains and to transfer by mosquitoes have thus far been unsuccessful. When serum from birds carrying the gametocyteless strain was mixed with parasites of the typical strain no effect on gametocyte production occurred. Likewise, serum from birds with typical infections did not stimulate gametocyte production when mixed with parasites lacking gametocytes.

Infections with the gametocyteless strain have been found to retain their a typical characteristics after a latent (or subpatent) period of three and one-half years.

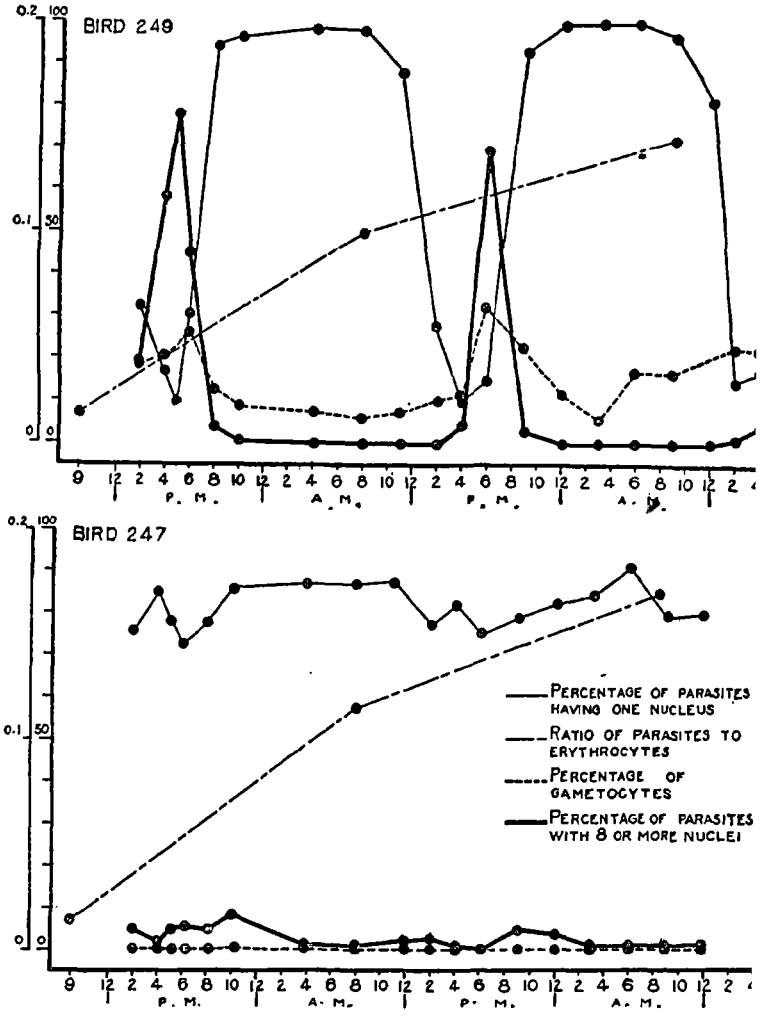


FIGURE 28—Gametocyte production and periodicity of reproduction in birds infected with the typical (249) and gametocyteless (247) strains of *P. cathemerium* during the beginning of the acute stage (from Huff and Gambrell, 1934).

4. EXFLAGELLATION AND FERTILIZATION

The release of mature male gametes from the male gametocyte either in drawn blood or in the stomach of the mosquito is known as *exflagellation.* This was first described in avian plasmodia by Danilewsky (1885) in his original observations on bird malaria, and was repeatedly mentioned in most of his subsequent publications. He regarded the flagellated organisms as separate parasites, and called them *Polymitus avium.* Manson suggested in 1894 that *Polymitus* was really a spore stage of the malaria parasite which passed into the stomach of a blood-sucking insect, and was transmitted to another vertebrate host through drinking water. The attempts of workers in both the fields of human and avian malariology to explain the formation of flagellated bodies led to many heated discussions which were of course cleared up only after MacCallum (1897) and Ross (1898) demonstrated their true purpose. A detailed discussion of the various theories which were suggested is not within the scope of the present work. MacCallum's description of the process in *Haemoproteus* is worthy of repeating, however, since an exactly similar situation occurs in all plasmodia. He describes exflagellation as follows:

"I decided, however, on the impulse of another idea, to observe carefully in the same field a granular form and a hyaline form from the time of extrusion from the corpuscle to the beginning of the motile stage, and, having found such a field, the following picture presented itself: The two forms lay at some distance from one another separated by the plasma and a few corpuscles. The granular form happened to escape from the corpuscle first and lay perfectly quiet beside the free nucleus and the shadow of the corpuscle. Soon the hyaline body, becoming greatly agitated, burst from the corpuscle, and threw out active flagella, which beat about for a few moments and finally tore themselves loose. Then came the acme of the process. One of the four flagella passed out of the field, but the remaining three proceeded directly toward the granular form, lying quietly acrosse the field, and surrounded it, wriggling about actively. One of the flagella, concentrating its protoplasm at one end, dashed into the granular sphere, which seemed to put out a process to meet it, and buried its head, finally wriggling its whole body into the

organism, which again became perfectly round. The remaining flagella, seeking to repeat this process were evidently repulsed, and soon became inactive and degenerated. Immediately on the entrance of the flagellum the pigment of the organism was violently agitated, without, however, any disturbance of the outline of the organism. Soon all became quiet again and the period of quiescence lasted for about fifteen minutes, when a conical process began to appear at one margin of the organism, which increasing in size, drew into itself most of the protoplasm, the pigment to a certain extent being gathered into the remainder. Finally most of the pigment was concentrated into a small round appendage, which remained attached to the end of what now had become an elongated fusiform body, which swam away with a gliding motion."

Succeeding investigations have added only detail to MacCallum's description of the fertilization process, and little work has been done on the maturation processes which probably occur in both the male and female gametocytes before mature gametes are produced. The liberation of flagellated male gametes sometimes occurs within a very few minutes after blood containing gametocytes is placed in saline solution, and the division of the gametocyte nucleus must occur exceedingly rapidly in order to liberate 6 or 8 gametes, each of which contain a portion of the original nucleus. Maturation in the female gametocyte would likewise need to be rapid. An interesting cytological problem is open for investigation here.

Raffaele (1939) has recently pointed out that the mode of production of microgametes in both avian and human plasmodia seems to confirm the existence of a sheath or capsule enveloping the microgametocyte. Before gametes are produced a projecting mass of cytoplasm containing most of the nuclear chromatin appears on the surface of the gametocyte and the gametes are pushed out from this mass.

5. THE DEVELOPMENT OF OÖCYSTS AND SPOROZOITES

After blood containing gametocytes is ingested by susceptible mosquitoes the process of exflagellation and fertilization described above occurs in the stomach cavity, and the

oökinete or motile zygote is formed. The length of life of the asexual forms in the stomachs of mosquitoes is said by Huff (1927) to be between 5 and 6 hours. The motile oökinete eventually finds its way to the stomach wall and penetrates the wall by passage between the cells of the stomach epithelium, as shown by Huff (1934). He states that the penetration of the stomach wall is not by a boring process, but is achieved by gradually forcing two of the stomach cells apart, as shown in figure 29.

When the zygote has reached its position under the outer

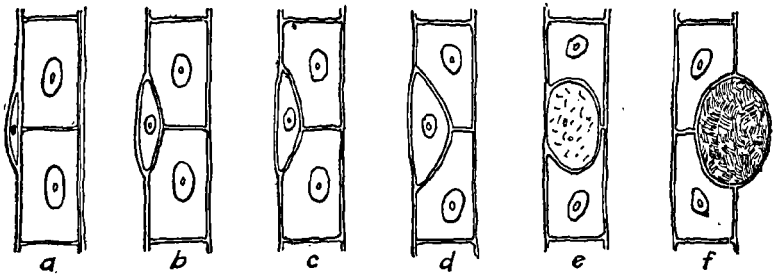


FIGURE 29—Diagram to represent the manner of passage of the oökinete through the stomach wall of the mosquito. The left side corresponds to the inner wall (from Huff, 1934).

envelope of the stomach (the *tunica elastica-muscularis*), an oöcyst is produced, which grows increasingly larger and within which sporozoites develop. Corradetti (1937) describes the formation of sporozoites in oöcysts of *P. circumflexum* as proceeding from furrows and complicated ridges towards the inner parts of the oöcyst; it is along these furrows or ridges that the sporozoites are found.

The size of oöcysts produced by *P. cathemerium, P. relictum,* and *P. elongatum* is given by Huff (1927), and his calculations show that the latter two species produce oöcysts significantly greater in size than those of the former species.

From the results of various workers, apparently from 7 to 10 days are required for complete sexual development to take place in the mosquito, that is to say, from the time of

fertilization to the liberation of sporozoites into the hemocoele from the oöcyst and their subsequent penetration into the salivary glands.

B. Mosquito Transmission and Epidemiology

1. HISTORICAL

The first transmission experiments with bird malaria were performed by Ross (1898), who described the exogenous stages of what was probably *P. relictum* or *P. cathemerium* in *Culex quinquefasciatus*. This work conclusively demonstrated that the vector of bird malaria was a mosquito, and the experiments which immediately followed on human malaria confirmed the discovery. Perhaps no other one piece of research in bird malaria has had such broad application to malaria in man. Ross's description of his first success in achieving development of avian plasmodia in the mosquito is as follows:

" In February, 1898, I was placed by the Government of India on special duty to prosecute these researches and was given the use of Surgeon-Lieutenant Colonel D. D. Cunningham's laboratory in Calcutta for the purpose. Steps were immediately taken to find again, if possible, the pigmented cells by feeding different species of mosquitoes on blood containing different species of gymnosporidia, . . . As it was not the malarious season of the year, and as cases of haemamoebiasis suitable for these experiments were not easy to procure, it was thought advisable to commence work on the so-called malaria parasites of birds. Accordingly a crow (*Corvus splendens*) and two tame pigeons infected with *Halteridium* were obtained; and on the night of March 11th and 12th these, with four short-toed larks (*Calandretta dukhunensis*) and six sparrows (*Passer Indica*), whose blood had not yet been carefully examined, were placed in their cages, all within the same mosquito netting, and a number of grey mosquitoes of the species I had lately been experimenting with were released within the net. Next morning numbers of these insects were found gorged with blood and were caught in test-tubes in which they were kept alive for two or three days.

On March 13th I commenced to examine them. Out of fourteen of them pigmented cells were found in at least one. Believing as I

did that these cells are derived from the gymnosporidia I judged from this experiment that the grey mosquito which now contained them had fed itself on one of the birds which happened to be infected by a parasite capable of transference to the grey species of mosquito. As all the birds had been placed together in the same net the question now was which one of them had the mosquito fed herself upon. This could easily be ascertained. A number of mosquitoes of the same species had meanwhile been fed separately on the crow and two pigeons with *Halteridium*; but out of thirty-four of these examined not one contained pigmented cells. Hence I came to the conclusion that the mosquito with pigmented cells had not derived them from the crow and two pigeons. The larks and sparrows remained. The blood of these had not yet been carefully searched. I now found that three of the larks and one of the sparrows contained proteosoma (Labbé) and therefore thought it possible that the mosquito had been infected from one of these. Accordingly, on the night of March 17th and 18th a number of grey mosquitoes were released on the three larks with proteosoma and next morning it was found that nine of these had fed themselves. On the morning of March 20th—that is, from forty-eight to sixty hours after feeding—these nine insects were examined. Pigmented cells were found in no less than five of them. After the long-continued negative experiments with this kind of mosquito (and, indeed, I may say, after three years doubtful attempts to cultivate these parasites) this result was almost conclusive."

Daniels (1899) and James (1902) likewise obtained development in *Culex quinquefasciatus* and were able to transmit the parasite (*P. relictum?*) from one bird to another. Koch (1899) demonstrated that avian plasmodia could also develop in *Aedes communis,* and Ruge (1901) and the Sergents (1905b) added *Culex pipiens* to the fast-growing list of susceptible mosquitoes. Further transmission experiments are referred to in table 21, in which a list of mosquitoes known to be capable of transmitting bird malaria is given.

2. REARING AND FEEDING MOSQUITOES

The following detailed account given by Huff (1927) is representative of one of several techniques used by investigators for rearing and feeding mosquitoes in the laboratory:

TABLE 21

SPECIES OF MOSQUITOES WHICH HAVE BEEN SHOWN TO BE SUSCEPTIBLE TO INFECTION WITH BIRD MALARIA PARASITES

Species of Mosquito	Species of Parasite	Author
Aedes aegypti Linn.	*P. relictum*	Sergents, 1906
	P. cathemerium, P. relictum	Huff, 1927
	P. gallinaceum	Brumpt, 1936
Aedes albopictus Skuse	*P. gallinaceum*	Brumpt, 1936
Aedes communis de Geer	*P. relictum*	Koch, 1899
Aedes geniculatus (Olivier)	*P. gallinaceum*	Roubaud *et al* (1939)
Aedes mariae Sergent	*P. relictum*	Sergents, 1918
Aedes sollicitans Walker	*P. cathemerium*	Herman, 1938
Aedes triseriatus Say	*P. elongatum*	Huff, 1932
Anopheles strodei Root *	*P. cathemerium*	Lucena, 1938
Anopheles subpictus Grassi	?	Mayne, 1928
Culex (*Lutzia*) *fuscana* Edw.	*P. cathemerium, P. relictum*	Nono, 1932
Culex hortensis Fic.	*P. relictum*	Sergents, 1918
Culex quinquefasciatus Say	?	Ross, 1898
	?	Daniels, 1899
	?	James, 1902
	P. cathemerium, P. relictum	Huff, 1927
Culex pipiens Linn.	?	Ruge, 1901
	P. cathemerium, P. elongatum, P. relictum	Huff, 1927
	P. circumflexum	Reichenow, 1932
	P. rouxi	Sergents and Catanei, 1928, Huff, 1932
	P. relictum	Raffaele, 1932
Culex salinarius Coq.	*P. cathemerium, P. relictum, P. elongatum*	Huff, 1927
Culex tarsalis Coq.	*P. cathemerium, P. relictum, P. rouxi, P. elongatum*	Huff, 1927
Culex territans Walker	*P. cathemerium, P. relictum, P. elongatum*	Huff, 1927
	P. rouxi	Huff, 1932
Theobaldia annulata Macq.	*P. circumflexum*	Reichenow, 1932
Theobaldia longiareolata Macq.	*P. relictum*	Sergents, 1918
Theobaldia melaneura Coq.	*P. circumflexum*	Herman, 1938

* Only one oöcyst found.

" Collections of larvae or pupae were made in the field. These were brought into the laboratory and placed in white bowls about 5 inches in diameter and 2½ inches deep, and the pupae were removed daily. The food which was found most satisfactory was Loeffler's dehydrated blood serum. This was sprinkled upon the surface of the water in small amounts twice daily. A few bread crumbs were usually placed in the water also. It was found necessary with most of the species to change the water at least every two days. Another precaution which was necessary was to avoid overcrowding of the larvae in the bowls. Fifty larvae to the bowl were considered the maximum number for good results.

As the pupae appeared they were removed and placed in crystallizing dishes 9 cm. in diameter and 5 cm. deep. A piece of cork was floated upon the surface of the water in these dishes, and a ring of dark paper, just large enough to cover the outside wall, was slipped over each. The cork gave the newly-emerged adults a place upon which to rest until they were ready to fly. The paper cover prevented unnecessary movements among the pupae by excluding most of the light and consequently cutting off the shadows from moving objects in the room. An ordinary lantern globe covered at the top with crinoline gauze was placed over the crystallizing dish. These globes were slightly less than 9 cm. in diameter at the bottom, so that they fitted snugly into the crystallizing dishes.

The pupae were allowed to remain in the emergence chambers described above until the adults had emerged. It was an easy operation then to remove the adults with the lantern globe by inserting two cardboards between the globe and crystallizing dish, and removing the globe with the upper cardboard. These globes were placed over Petri dishes filled with moistened " cellucotton," and set aside until ready to be given their blood meal. Soaked raisins were placed on the netting covering the top of the globe to serve as food and drink. During the hottest weather of summer I found it advisable to use candied raisins which could be moistened frequently, but which would not ferment.

A great deal of difficulty was encountered at first in getting the adult females to feed upon the birds. Two factors were essential for success in this attempt. It was found necessary to keep the adults away from water for at least twenty-four hours before attempting the feeding experiment. During extremely hot weather it was necessary to keep the breeding cages over moistened cotton so as to keep the air moist, but in such a way that the mosquitoes could not imbibe the liquid. The other important factor was darkness. Nearly all

species dealt with in these experiments would bite much better in darkness than they would in the light. Hence nearly all of the feedings were made at night.

In order to allow the mosquitoes to feed, the feathers of the bird were parted in the pectoral region and wetted down. The bird was then immobilized by tying it snugly in a piece of netting, and was placed upon the top of the breeding chamber in such a way that its exposed breast would be accessible to the mosquitoes. A strip of cloth was placed over it and extending down along the side of the lantern globe and a rubber band placed around the globe in such a way as to hold the strip firmly in position. The bird could be left in this position for at least an hour without apparent discomfort.

When the female mosquitoes had engorged upon the blood of the infected bird, the bird was removed and all of the mosquitoes in this breeding chamber were liberated into a catching bag made of netting and provided with a long sleeve. It was then an easy matter to pick out the engorged females individually and to transfer them to another breeding chamber. In so doing it was necessary to wear a rubber glove to prevent the mosquitoes from engorging from my hand; for they might then be mistaken for mosquitoes which had had the infective meal.

The fed mosquitoes were kept from eight to twelve days and dissected. It was soon found, under the prevailing laboratory conditions, that the oöcysts of the parasite usually reached their maximum size on the tenth day following the feeding. Therefore, the mosquitoes were kept thereafter for ten days as a matter of routine. After they had been anaesthetized, each of the mosquitoes was identified before being dissected. In the case of the species which showed the presence of oöcysts, a few were kept long enough to insure the finding of sporozoites in their salivary glands. No attempt at counting the number of oöcysts upon the stomachs were made, since this count could in most cases have been only an estimate, due to the great number present and the difficulty of viewing all sides of the stomach without duplicating the count of certain oöcysts."

In a later paper by the same author (Huff, 1930b) it is mentioned that mosquitoes were kept at all times in a warm room, electrically controlled and heated at 79° F.–82° F. (26.0° C.–27.7° C.).

Variations of the above procedure are practiced by different workers, depending upon the size of the colony to be

maintained and the facilities available for handling mosquitoes. In this laboratory the adults are reared in square, bobbin-netting cages with board bases and wire supports. A sleeve is attached to one side for facilitating the admission of birds and withdrawing mosquitoes. When mosquitoes are to be fed, the bird is placed in a small wire cage to prevent excessive movement and the cage is placed in the bobbin-netting overnight. The feathers are previously clipped from the head of the bird. By the following morning many of the mosquitoes are usually engorged with blood; these can be removed to a special cage.

A constant temperature and humidity chamber is desirable if colonies of mosquitoes are to be kept over long periods of time. This can be constructed according to the specifications desired, and lined with shelves properly spaced for the accommodation of bobbin-netting cages. The temperature can be electricaly controlled by a thermostat and pans of water placed in the bottom will supply the proper degree of humidity.

Difficulty is frequently encountered in getting the mosquitoes to take a second blood meal. Huff (1930b) reports that out of a total of 678 mosquitoes fed once, only 176 (25.96 per cent) could be induced to feed a second time. Some died immediately after depositing eggs, others drowned in the water tray, and some died shortly after the first feeding before the blood meal was digested. Many refused to feed a second time although they were given opportunities to do so. It is necessary, then, in order to insure enough mosquitoes for successful transfers, that considerable allowance be made for the difficulties involved in carrying the transmission to completion.

The average weight of *Culex pipiens* has been found by Shah, Rozeboom and Del Rosario (1934) to be 1.4 milligrams, and the average weight of the blood ingested by this species is 1.2 milligrams.

Not all stages of infection in the vertebrate host are suitable for feeding mosquitoes if sexual development is to be assured. To be satisfactory in this respect a high percentage

of gametocytes must be present in the blood of the bird. Huff (1930b) points out that only long continued experience with feeding mosquitoes will enable one to distinguish those infections which are suitable for feeding experiments.

Et. Sergent (1919a) states that the optimum temperature for the development of avian plasmodia in the mosquito is from 20° to 30° C. A temperature of 12° C. for the first 6 hours after a blood meal does not affect the development in the mosquito. After 6 hours at this temperature, however, the parasites will not complete their development.

3. DOUBLE FEEDINGS TO INCREASE CHANCES OF INFECTION

A certain percentage of the mosquitoes which are fed on parasitized birds will fail to become infected, even though other mosquitoes fed on the same bird exhibit full development of the parasites. Huff (1930b) felt that double feedings might increase the chance of infection, and performed a series of experiments to test this point. Of fifteen mosquitoes fed twice on *P. relictum* infections all were infected by the second feeding, and all but two were infected by the first feeding. When fed upon *P. elongatum* infections all of 20 mosquitoes were negative after both the first and second feeding. In the latter case, however, it was previously shown that an infection rate of 3.2 per cent occurred when this species was used. The relationship between these results and natural immunity in the mosquito will be discussed later, but it appears for practical purposes that second feedings do not appreciably increase the chance of obtaining infections.

4. THE PERIOD DURING ASEXUAL PATENCY BEST FOR INFECTING MOSQUITOES

It has already been pointed out that a successful infection in the mosquito depends on the ingestion of an adequate number of male and female gametocytes. Since the number of gametocytes increases as the infection in birds progresses

to the crisis, it appears that better results will be obtained if mosquitoes are fed during the peak of gametocyte production. Shah, Rozeboom and Del Rosario (1934) came to the following conclusions when they compared the number of gametocytes ingested and the percentage of infections obtained in the mosquitoes;

(a). In mosquitoes fed on birds when no gametocytes per 500 leucocytes were detected in the peripheral blood, less

TABLE 22

A COMPARISON OF THE NUMBER OF GAMETOCYTES INGESTED BY MOSQUITOES AND THE PERCENTAGE OF MOSQUITOES WHICH BECOME INFECTED (FROM SHAH, ROZEBOOM AND DEL ROSARIO, 1934)

	Number of gametocytes ingested by mosquitoes							
	None *	1-25	26-1,000	1,001-5,000	5,001-10,000	10,001-50,000	Over 50,000	Total
Mosquitoes								
Positive	1	1	3	20	9	10	2	46
Negative	10	3	3	10	1	0	0	27
Total	11	4	6	30	10	10	2	73
Per cent positive.	9.9	25.0	50.0	66.7	90.0	100	100	

* No gametocytes observed per 500 leucocytes.

than 10 per cent became positive. Only 25 per cent showed infection when they had ingested less than 25 gametocytes.

(b). With the ingestion of increasing numbers of gametocytes the rate of infection increased until 100 per cent of the mosquitoes became positive. Table 22 illustrates the number of infections obtained following the ingestion of the designated number of gametocytes.

It was found by the same authors that some mosquitoes became infected when fed on birds during the first and second days of asexual patency, but when fed on old subpatent cases no infection occurred.

5. THE COURSE OF INFECTION IN THE VERTEBRATE HOST FOLLOWING INJECTIONS OF SPOROZOITES EITHER ARTIFICIALLY OR BY MOSQUITOES

The Sergents (1912) found the prepatent period in the vertebrate host to be from 6 to 9 days following mosquito transmission of the parasite (*P. relictum*). This was substantiated by Huff (1927, 1932) in infections with the same species of parasite. Herman (1938b) states that the prepatent period following mosquito transmission of *P. circumflexum* and *P. cathemerium* to redwing blackbirds and cowbirds is from 8 to 14 days. Further experimental data of this nature are needed for all species of bird malaria parasites before a general statement can be made which will include variations which may occur within species. It appears, however, that the prepatent period following mosquito inoculation is somewhat longer than that which occurs after direct blood inoculation.

Sporozoites taken directly from the salivary glands of infected mosquitoes will produce infections in birds when they are inoculated intravenously (Wolfson, 1936b; Warren and Coggeshall, 1937), intramuscularly (Rozeboom and Shah, 1934; Warren and Coggeshall, 1937), and intraperitoneally (Raffaele, 1934b). Infections are also produced when birds are inoculated with sporozoites taken from stomach oöcysts or from the peritoneal cavity of mosquitoes (Neri, 1938). The method usually used to transfer sporozoites artificially is to mix them with Locke's physiological saline and inject with a hypodermic syringe. The Sergents (1912), however, produced infections in 4 of 10 canaries by rubbing the crushed thorax of infected mosquitoes into a scratch on the exposed skin of the birds. Rozeboom and Shah (1934) state that 200 or more sporozoites must be introduced into canaries in order to produce infections with *P. cathemerium.*

The prepatent period in canaries following the introduction of sporozoites into a scratch on the thorax, as observed by the Sergents (1912), varied from 6 to 9 days (*P. relictum*). Wolfson (1936b) first found parasites in the vertebrate host 10 days after intravenous injections with sporo-

zoites. Gametocytes appear coincidentally with the appearance of asexual stages following sporozoite injections (Shah, Rozeboom, and Del Rosario, 1934).

6. STUDIES ON IMMUNITY IN THE MOSQUITO

In attempts to discover why certain individual mosquitoes are refractory to infection with malaria parasites, and also why complete exogenous development will take place in other species of mosquitoes, Huff (1927, 1929, 1930, 1931, 1932, 1934) conducted a series of experiments on the natural immunity and susceptibility of culicine mosquitoes to bird malaria. In his first paper (1927) he reported that *P. cathemerium* could develop to the oökinete stage in *Aedes sollicitans* (an insusceptible species) as well as in *Culex pipiens* (a susceptible species). No differences were observed with regard to the effects of digestion on the red cells and parasites in the stomachs of either of these species. Individual immunity was observed within *Culex pipiens*, in that certain mosquitoes of this species could not be infected with *P. cathemerium, P. relictum,* or *P. elongatum.* Furthermore, this immunity was found in later experiments (1930b) to be parasite specific, in that an individual mosquito immune to one species of *Plasmodium* was not always immune to another species. Double infectious feedings on the same species showed that mosquitoes either became infected at both feedings or resisted infection altogether.

In experiments on the effects of selection and inbreeding (1929) as well as in studies of the geneology of certain races (1931) he demonstrated a rapid increase in the percentage of infected individuals among the offspring of inbred susceptible females (figure 30). Susceptibility proved to be a Mendelian recessive character (figure 31), but none of the results indicated sex-linkage.

Histological studies (1934) made upon the blood meal, the condition of the stomach wall, and the appearance of zygotes and oöcysts in susceptible mosquitoes showed no evidence of cellular response in the latter, strengthening the hereditary nature of the phenomena of susceptibility and insusceptibility in individuals and species of mosquitoes.

Although confirmatory studies of a similar nature have not been made with bird malaria or human malaria, Huff's comprehensive treatment of the subject leaves little doubt that at least one of the important factors which determines

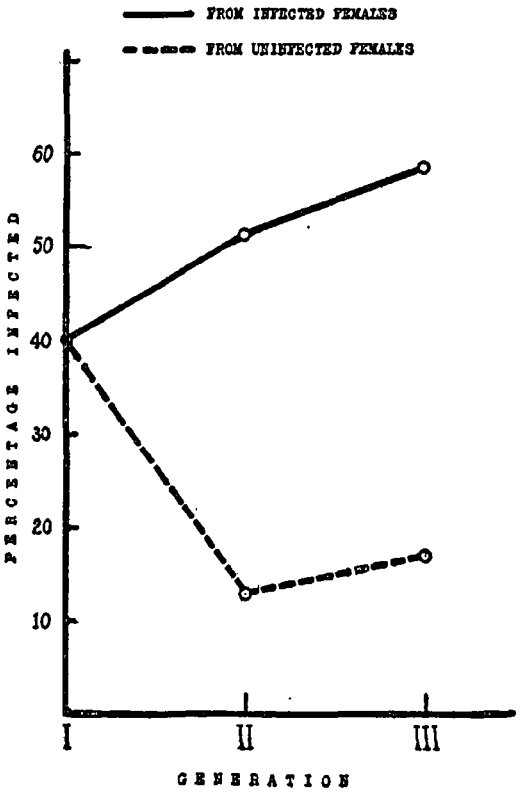


FIGURE 30—The effects of selection upon susceptibility in *Culex quinquefasciatus* to *P. cathemerium* (from Huff, 1931).

the susceptibility of certain mosquitoes to malaria parasites is the hereditary background of the particular individual or species in question.

Roubaud and Mezger (1934) have suggested that mosquitoes which are constantly exposed to bird malaria infection (e. g. rural strains of *Culex pipiens*) acquire a certain

immunity which is entirely absent in the races (urban strains) which rarely, if ever, feed on birds. They used 3 races of *Culex pipiens*; one urban strain could be regularly infected with *P. relictum*, and in two rural strains infections occurred less frequently.

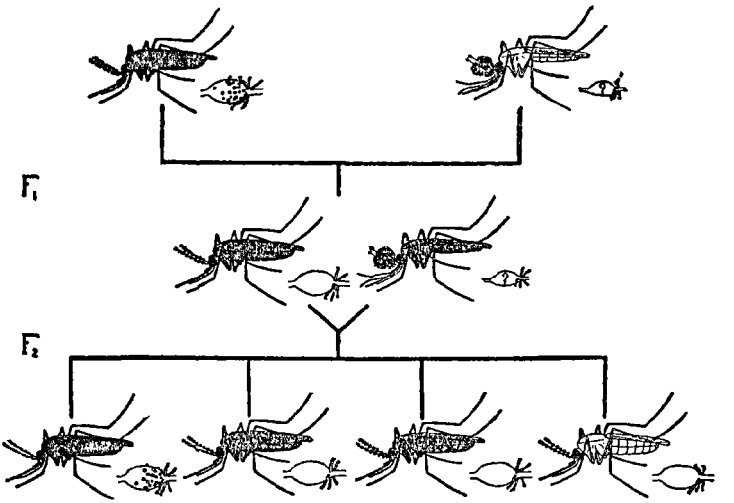


FIGURE 31—Diagram to illustrate the inheritance in *Culex pipiens* to *P. cathemerium*. Black represents the homozygous condition of susceptibility, white, the homozygous condition of insusceptibility, and barred, the heterozygous condition. Males of the F_2 generation are omitted since the genotypic organization is similar to that of the females (from Huff, 1935).

7. THE EFFECT OF THE PARASITES ON THE INVERTEBRATE HOST

Apparently only two attempts have been made to determine the effect of avian plasmodia on the mosquito. Et. Sergent (1919b) observed hundreds of infected mosquitoes throughout a long period of time and never found a higher mortality rate in infected mosquitoes than in non-infected ones. He therefore concluded that *P. relictum* is not pathogenic for *Culex pipiens* and does not seriously affect the

insect. Buxton (1935), on the other hand, found that deaths occurred earlier in mosquitoes infected with *P. relictum* than in non-infected controls. At 30° C. the effect of the infection in the mosquitoes was apparent on the 1st day after the blood meal, and at 23° C. deaths occurred as early as the 2nd day after feeding. An increased number of deaths in the infected mosquitoes occurred on the 7th day after feeding, which, the author believes, is due to the growth of oöcysts.

These conflicting results indicate the need for further researches in this direction, since it is possible that certain species of parasites act differently from others in their effects on the life processes of the invertebrate host.

Sinton and Shute (1938) have recently reviewed the literature on human malaria relative to this subject and conclude that the evidence available adds little support to the suggestion that human plasmodia may be a serious cause of mortality among anopheline mosquitoes in nature.

8. EPIDEMIOLOGY

The first published report on the epidemiology of bird malaria in experimental laboratory animals seems to be that of Russell (1931e), although this author refers to an abandoned study of the subject with malaria in sparrows mentioned by Gill (1928) in his textbook on epidemiology. Russell used *P. cathemerium* as the experimental parasite, canaries as the vertebrate hosts, and *Culex pipiens* and *C. quinquefasciatus* as the vectors. Cages were constructed to harbor both the bird colonies and the mosquito colonies. In one experiment, after colonies of mosquitoes propagating strongly were established in the cages, a number of non-infected birds were place in the cage and a constant supply of gametocyte carriers was made available. One gametocyte carrier was kept in the cage with 9 susceptible birds, and fresh parasitized canaries were frequently substituted to insure a constant source of parasites. At the end of three months the experiment was terminated, and no transmission had occurred. Russell believes that the "epidemic potential"

was not high enough in this experiment to bring about transmission, the term epidemic potential meaning the balance of interacting forces which tends towards the occurrence of an outbreak of disease.

In another experiment, one susceptible bird was placed in a cage with three gametocyte carriers, plus mosquitoes. The carriers were substituted 15 times over a period of one month and at the end of that time the susceptible bird was infected. Here the " epidemic potential " was high, 80 per cent of the population in the cage being gametocyte carriers, and it was practically a foregone conclusion that the susceptible host would become infected.

This work, although not conclusive, presents another interesting use of experimental bird malaria infections. Modifications of Russell's procedures might give results which would have a direct bearing on similar situations which could presumably arise in human populations.

Herman (1938b) conducted an epidemiological investigation of *P. circumflexum* and *P. cathemerium* in red-wing blackbirds; this appears to be the only published work on transmission under natural conditions. Adult red-wings were captured in traps and the presence of plasmodia was determined by blood examinations or by subinoculations into uninfected canaries. Blood smears were also made of nestlings within a day or two before they left their nests. When the birds were recaptured, further smears and subinoculations were made as in the case of the adults. Of 53 adult birds examined, 60 per cent were infected with either *P. cathemerium* or *P. circumflexum.* No organisms were found in smears from 45 nestling birds before they left their nests. Twenty-six of these were recaptured after leaving the nest and 9 of these were found to be infected. The evidence suggests that the young birds did not obtain their infections while on the nest, but that some acquired infections after leaving the nest. The higher prevalence of malaria in adult birds was explained either as a result of yearly variations in incidence in young birds due to variations in the mosquito density, or due to an increase in infections following migration.

CHAPTER X

EXOERYTHROCYTIC STAGES ASSOCIATED WITH THE LIFE CYCLE

A. EXOERYTHROCYTIC SCHIZOGONY

1. HISTORICAL

Although recent papers by Huff and Bloom (1935) and James and Tate (1937) have aroused widespread interest in the occurrence of schizogonic stages in cells other than erythrocytes, or free in the tissues, this possibility was given consideration in some of the earliest researches on bird malaria parasites. It is perhaps overstepping proven facts to begin with the premise that such stages do occur in the development of plasmodia, since there is still some doubt as to whether the life cycle of some other parasite is not involved. However, the constant association of exoerythrocytic stages with certain strains of bird malaria, and the negative results thus far obtained in attempts to separate these stages from the more familiar cycle in the red blood cells, leads to the belief that they may represent a stage in the asexual development of the organisms.

In searching Danilewsky's publications, but one reference has been found which, although very obscure, may refer to schizogony outside of erythrocytes. In 1890 (c) this writer compared the plasmodia of birds and man and came to the conclusion that they were the same parasites. Referring to the appearance of "free spores" (probably merozoites which had escaped from red blood cells) in infected birds, he states that they frequently resembled the spores of *Sarcosporidium* or *Microsporidium*. The picture immediately brought to mind is the large masses of elongate or oval spores found in the tissues in infections with these two genera. Whether Danilewsky was referring to single merozoites which were free in the plasma or whether he was describing masses of small

parasites is difficult to say. It must also be remembered that for the most part he was probably dealing with mixed infections of *Haemoproteus* and *Plasmodium*, so that his observation cannot be given too much credence for this reason. Ziemann's (1898) reference to exoerythrocytic forms in "*Proteosoma*" infections must also be treated with some skepticism since it is very probable that mixed infections were involved in his case. In the same year, however, MacCallum (1898a) observed division forms free in tissue and in the reticuloendothelial cells of the spleen and liver of birds infected with "*Haemamoeba.*" His description of these stages is as follows:

"Among these there are often seen cells containing small oval bodies just about the size of the nucleus of a red corpuscle. These bodies take a general lilac color and show in their centres a ring-shaped structure which stains rather deeply with haematoxylin. These ovoid structures may occur free in the midst of the debris of cells and nuclei, but are more often contained in cells, where they may be seen in groups of two or more, or in larger collections of from twelve to twenty arranged with the pointed end turned toward the center and calling to mind the appearance presented by the segmented bodies of a malarial parasite."

Pigment was not observed in these bodies. Illustrations were given, one of which is reproduced in plate X, figure 5.

The next investigator to report exoerythrocytic parasites in association with *Plasmodium* infections in birds was Laveran (1900), who found them in Java sparrows (*Padda oryzivora*). This report differs from that of MacCallum since the parasites described always occurred within reticulo-endothelial cells, and in no case were division stages positively identified. They were spherical or oval in shape, 2 or 3 micra in diameter, and occurred either in a depression within the nucleus of the host cell or sometimes within the interior of the nucleus. None were encountered in the liver, and more occurred in the spleen than in the bone marrow. Laveran suggested that these bodies might represent another stage in the development of bird malaria parasites, but was careful to include the notation that more work would have to be done

before definite conclusions could be drawn. Figures were not given, but from the description it seems quite certain that the bodies described represent a type of parasite entirely different from that mentioned by MacCallum. Surprisingly few references occur in subsequent publications to either MacCallum's or Laveran's introduction into the literature of parasites which might add an additional cycle to the known asexual stages of plasmodia.

A new complication arose in two papers by Anschütz (1909, 1910) on the life cycle of *Haemoproteus oryzivorae* in the vertebrate host. Both intracellular and extracellular schizogony was described and pictured for this parasite, although it is quite clear that the writer was dealing with mixed infections of *Plasmodium* and *Haemoproteus*. He mentions this himself in the second paper, and illustrates what he called division of the macrogametocyte in the first paper, but the latter were undoubtedly segmenters of *P. relictum* or *P. cathemerium*. Large masses of segmenting forms are pictured in the blood vessels; these compare favorably with the stages described almost 30 years later by James and Tate (1937) in infections with *P. gallinaceum*. Some of Anschütz's illustrations are reproduced in plate X (figures 4 and 11) to show the similarity between the exoerythrocytic schizonts which he found and those described by James and Tate.

Although it can never be conclusively demonstrated that the division forms which Anschütz found were actually exoerythrocytic stages of *Plasmodium* (since *Haemoproteus* was also present) his papers are among the first to present the possibility that large schizogonic masses located free in the tissues or in blood vessels may represent an additional method of reproduction in the bird malaria parasites.

Twenty-five years passed before the question of exoerythrocytic stages in bird malaria were again referred to. Raffaele (1934a) and Huff and Bloom (1935) reopened the problem by describing the complete development of *P. elongatum* in white blood cells and reticulo-endothelial cells, as well as in red blood cells. Figure 32 (from Huff and Bloom)

shows the complete development of the asexual cycle in hemocytoblasts, and it is pointed out by the writers that the same cycle occurs in all of the other blood and blood-forming cells (plate XI). These investigators seemed to prove defi-

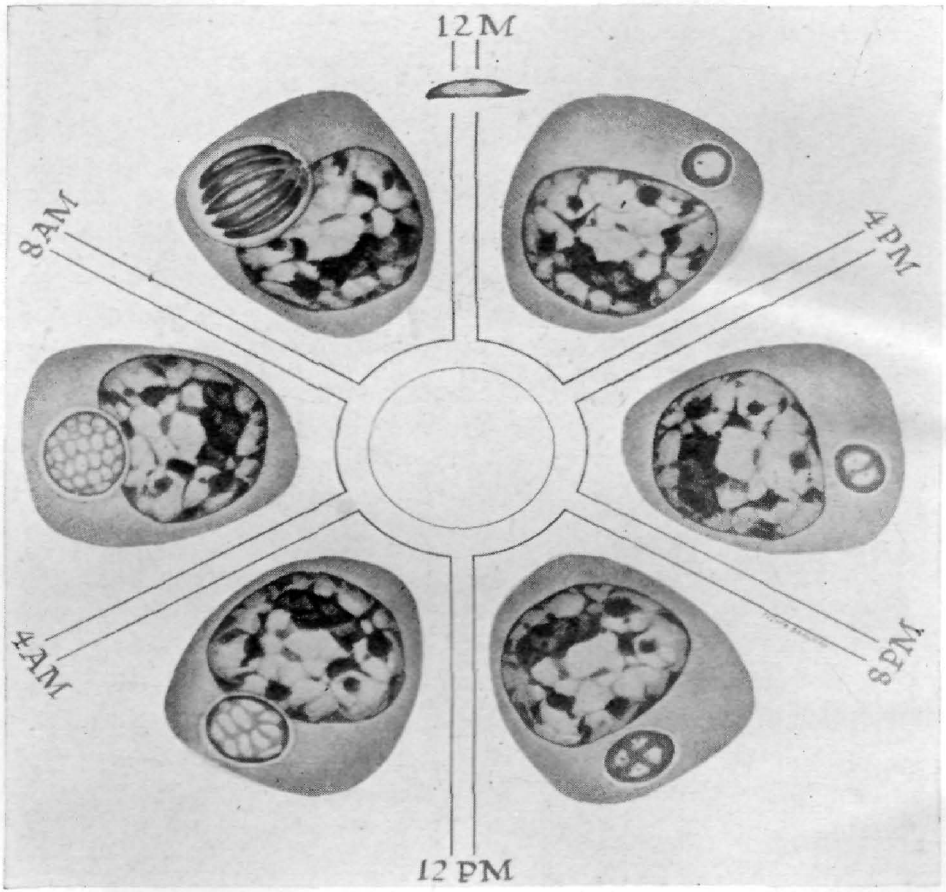


FIGURE 32—Diagram of the asexual cycle of *P. elongatum* in hemocytoblasts (from Huff and Bloom, 1935).

nitely that the asexual cycle of at least one species of bird *Plasmodium* could occur in cells other than erythrocytes, although some of the stages pictured are unlike any other known forms of bird malaria parasites.

Raffaele (1936b) found similar stages in *P. relictum* infections, and in 1937 summarized his observations on the

PLATE IX—A drawing of an autopsy performed on a canary infected with a strain of *P. cathemerium* which is regularly accompanied by exoerythrocytic bodies. The spleen lies at the far right and is somewhat larger than that which occurs in the usual laboratory infections with this species. It measured 26 mm. long by 9 mm. wide. The spleen of an uninfected canary is about the size of a grain of wheat. (Reproduced through the courtesy of Dr. Fruma Wolfson.) (x1).

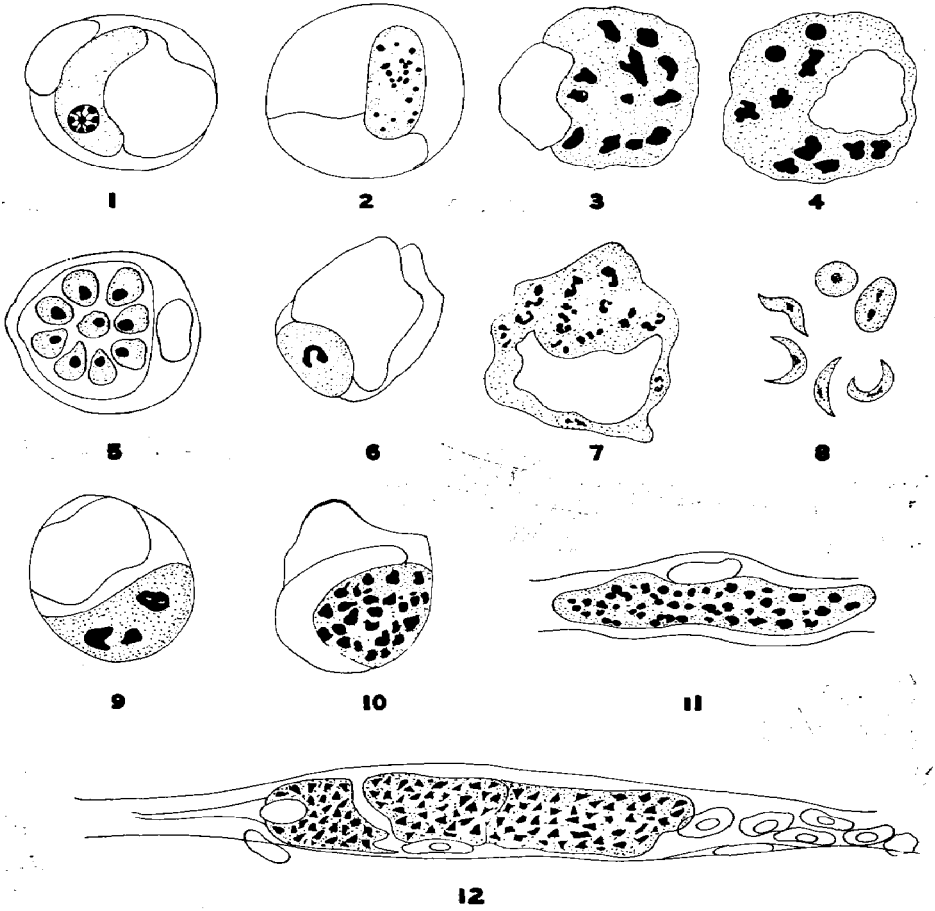


PLATE X—Types of exoerythrocytic parasites which have been found in birds (1-10, x1800; 11-12, x700).

Figure 1. *Haemogregarina atticorae*(?) within a white blood cell (after Aragão, 1911).

Figure 2. *Haemogregarina poroariae*(?) within a white blood cell (after Aragão, 1911).

Figure 3. Schizogonic stage of *Haemogregarina sporophilae*(?) within a white blood cell (after Aragão, 1911).

Figure 4. Schizogonic stage of *Haemoproteus oryzivorae*(?) within a white cell from the spleen (after Anschütz, 1909).

Figure 5. Segmenting form in a white blood cell from the liver in an infection with *Plasmodium* and *Haemoproteus*(?) (after MacCallum, 1898).

Figure 6. *Toxoplasma*(?) in a leucocyte from the peripheral blood of a chipping sparrow (after Herman, 1937).

Figure 7. *Toxoplasma*(?) in a leucocyte from the liver of an English sparrow (after Herman, 1937).

Figure 8. Extracellular *Toxoplasma*(?) from the liver of an English sparrow (after Herman, 1937).

Figure 9. A monocyte from the liver of a chick, showing a growing schizont with 3 chromatin masses (*P. gallinaceum*) (after James and Tate, 1938).

Figure 10. A monocyte from the lung, containing a large schizont with 25 chromatin masses, some of which appear to be dividing (*P. gallinaceum*) (after James and Tate, 1938).

Figure 11. A portion of a brain capillary showing schizogony of *Haemoproteus oryzivorae*(?) (after Anschütz, 1910).

Figure 12. A brain capillary of the chick containing 3 large schizonts (*P. gallinaceum*) (after James and Tate, 1938).

occurrence of *P. elongatum* and *P. relictum* in reticulo-endothelial cells.

Herman (1937c) described as *Toxoplasma paddae* a number of different types of exoerythrocytic parasites from wild birds, either in combination with *Plasmodium* or from birds seemingly free from malarial infection (plate X, figures 6, 7 and 8). The description of these parasites will be taken up later, since, although some of the stages represented resemble those described by Huff and Bloom (1935), as well as by James and Tate (1937), the nomenclature in this case is undoubtedly confused.

The report by James and Tate (1937) of a "new" life cycle in *P. gallinaceum* infections seemed to arouse more widespread interest than any of the preceding papers on the subject, probably because both of the writers were medical men and their description of exoerythrocytic stages in malaria appealed directly to those interested in the possibility that a similar cycle might be found in human malaria. Unpigmented, schizogonic masses were found in endothelial cells of the spleen and heart blood (plate X, figures 9 and 10), and large schizonts were described in the endothelial cells lining the capillaries of the brain (plate X, figure 12). Photographs of preparations from the heart blood, spleen, liver, and brain were presented in a paper which immediately followed, and all of these showed different stages of schizogony in endothelial cells.

Brumpt (1937) and Giovannola (1938) confirmed this work, and similar stages were found in infections with *P. cathemerium* (Kikuth and Mudrow, 1937, 1938; Hegner and Wolfson, 1938a), *P. circumflexum* (Manwell, 1938b; Manwell and Goldstein, 1938a and b), and *P. nucleophilum* (Hegner and Wolfson, 1938a). Raffaele (1936b) and Hegner and Wolfson (1938a) also describe exoerythrocytic parasites in *P. relictum* infections; the papers by Raffaele (1934a, 1936a) and Huff and Bloom (1935) on *P. elongatum* have already been mentioned.

That exoerythrocytic parasites are frequently associated with certain species and strains of avian plasmodia is defi-

nitely established, but all investigators are not in agreement regarding their significance. At least 3 different types have been described: (1) non-pigmented, segmenting forms in reticulo-endothelial cells of the spleen and liver (MacCallum, 1898a; Raffaele, 1934a; Huff and Bloom, 1935; James and Tate, 1937; etc.—plate X, figure 10), (2) oval or spherical parasites found either within the nucleus or in a concavity of the nucleus in reticulo-endothelial cells of the spleen and liver (Laveran, 1900; Herman, 1937c—plate X, figure 6), and (3) large schizogonic masses either free in the tissue or in endothelial cells lining the capillaries of the brain (Anschütz, 1909, 1910; James and Tate, 1937; Brumpt, 1937, and others—plate X, figure 12). Decourt and Schneider (1938b) describe still another type which is not definite enough to include in any of the above groups. These are small red to purple staining granules, 1 to 1.5 micra in diameter, which occur in the cytoplasm of lymphoid cells in the lymphoid tissue and the blood. By keeping malaria parasites (*P. gallinaceum*) in sodium citrate solution at 28° C., the parasites were seen to break up into daughter cells, and these, without leaving the cell became transformed into granules similar to the above-mentioned.

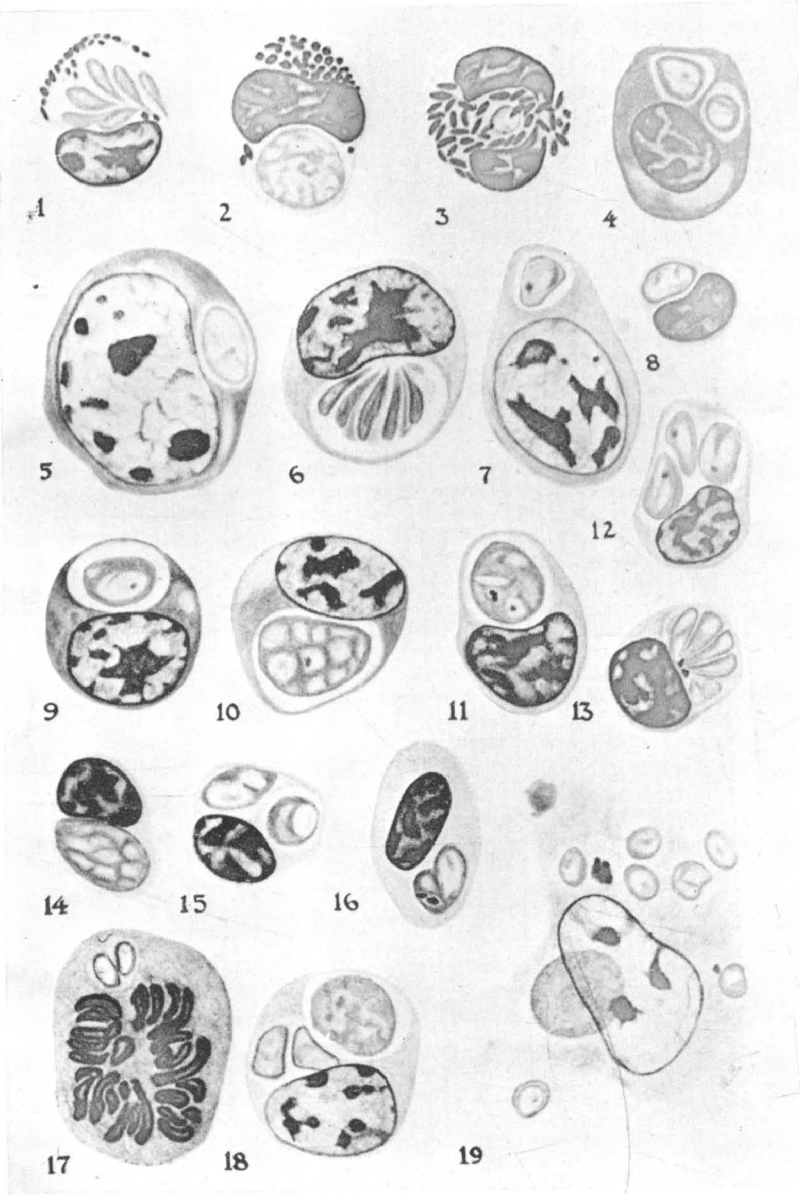
Certain stages in the life cycle of other species of blood parasites (*Haemoproteus, Leucocytozoon, Anaplasma, Toxoplasma*, etc.) resemble all of the types of exoerythrocytic schizogony described in bird malaria, and the possibility of mixed infections must therefore be considered, as pointed out by Hegner and Wolfson (1938a), and Manwell (1939). A discussion of the various possibilities in this connection will be included under some of the topics which follow.

2. CHARACTERISTICS OF INFECTIONS ASSOCIATED WITH EXOERYTHROCYTIC PARASITES

It appears that in all infections with *P. elongatum* asexual stages can be found in all types of blood cells (Raffaele, 1934a; Huff and Bloom, 1935). Certain stages of this parasite are different from most other species, particularly the

PLATE XI—*Plasmodium elongatum* in various types of blood cells from the bone marrow, spleen, and liver of infected canaries (from Huff and Bloom, 1935). (x2500).

1. Segmenter in young heterophil myelocyte. 2. Degenerating schizont in heterophil myelocyte. 3. Very young trophozoite in heterophil metamyelocyte. 4. Trophozoites in plasma cell. 5. Trophozoite in transition form between primitive reticular cell and hemocytoblast. 6. Segmenter in hemocytoblast. 7. Trophozoite in hemocytoblast. 8. Trophozoite in small lymphocyte. 9. Trophozoite in basophil erythroblast. 10. Schizont in very early polychromatophil erythroblast. 11. Schizont in early polychromatophil erythroblast. 12. Trophozoite in polychromatophil erythroblast. 13. Segmenter in polychromatophil erythroblast. 14. Schizont in normoblast. 15. Trophozoite in normoblast. 16. Trophozoite (or young gametocyte) in erythrocyte. 17. Young trophozoite in polychromatophil erythroblast in mitosis. 18. Two trophozoites and degenerating schizont in monocyte. 19. Seven young trophozoites in macrophage.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merozoites, which in moist fixed smears appear elongate rather than round or oval (see figure 32). Furthermore, segmenting stages rarely occur in the peripheral blood, and are localized in the spleen, liver, and bone marrow. Huff and Bloom have found that periodicity in the asexual cycle is manifested more strikingly in the peripheral blood than in the bone marrow, and that the cycle is quotidian. In most cases, the infections produced by *P. elongatum* are low grade and do not seem to seriously affect the bird. As far as could be determined by Huff and Bloom the occurrence of parasites within monocytes, myelocytes, plasma cells, and stem cells, as well as in erythrocytes (plate XI), seems not to interfere with the life of the host cells. The fact that all types of blood cells are invaded apparently does not make infections with this parasite more severe than if only erythrocytes were attacked. The host-parasite adjustments here seem to be well-balanced, and there is no evidence to indicate that the occurrence of the asexual cycle in all types of blood cells is not a normal occurrence.

In contrast to this situation, all other species of bird malaria associated with exoerythrocytic parasites show marked changes in virulence and injury to the host when such stages are exhibited. James and Tate discovered exoerythrocytic schizogony in a fowl which had survived the acute stage of the infection, when suddenly, on the 19th day from the onset of symptoms it became drowsy and appeared to be in a semicomatose condition, with ruffled feathers and closed eyes. Death occurred on the next day. Parasites were not found in the peripheral blood for several days preceding this sudden appearance of marked symptoms, but on the day of death the parasite count was very high (James, 1939). This marked rise in parasite number coincident with the appearance of exoerythrocytic parasites in the visceral organs has been noted by other workers (Wolfson, 1940a; Hewitt, 1940c).

In comparing the incidence of exoerythrocytic stages in *P. gallinaceum* infections produced by sporozoite inoculations and by direct blood transfer, James (1939) makes the following statement:

" From these results it will be seen that in *P. gallinaceum* exoerythrocytic schizogony occurs commonly at a late stage of the disease contracted by the inoculation of either infective blood or sporozoites. Indeed, in cases which were fatal at this late period it was found invariably in both types of infection. In blood infections when it occurs during the 3rd week of the disease—as it so often does—it may almost entirely take the place of the ordinary type of schizogony in erythrocytes and by reason of its abundance and wide spread may bring about a fatal issue irrespective of the degree to which red cells are parasitized. In some other cases (as in relapses following splenectomy) schizogony in endothelial cells and in red blood corpuscles may proceed concurrently."

He furthermore found that exoerythrocytic bodies occurred earlier in infections following sporozoite injections than after direct blood transfer, and that infections following sporozoite injections were more fatal in the acute stage than after blood inoculations.

Manwell and Goldstein (1939a) found exoerythrocytic stages in 16 cases of *P. circumflexum* and in all but one they occurred in the acute stage of the disease.

The pathology in canaries which exhibit exoerythrocytic parasites seems to be more severe than in birds infected with the same strain of parasite but not showing exoerythrocytic bodies. Wolfson (1940a) has demonstrated this in infections with a strain of *P. cathemerium* isolated from a wood-thrush, although the length of life of canaries exhibiting exoerythrocytic bodies was not significantly less than those which did not show these stages. Infarcts and enlargements of the spleen and hemorrhages in the membrane of the brain among canaries dead in their cage occurred much more frequently in those which showed exoerythrocytic schizogony than in those in which such stages could not be found. Manwell and Goldstein (1939a) also state that a definite relationship probably exists between the pathogenicity of infections with *P. circumflexum* and the presence of exoerythrocytic stages in the visceral organs.

Two types of schizonts in the endothelial and reticuloendothelial cells are noted by James and Tate (1938) and Manwell and Goldstein (1939a). The difference between

them is the intensity of the cytoplasmic stain. One type stains very lightly and the other very deeply. Aragão (1908) and Anschütz (1910) described similar stages in *Haemoproteus* infections, and believed that they might represent schizonts which were to eventually become male and female gametocytes. Manwell and Goldstein point out, however, that the number of gametocytes in the circulating blood is usually much too small to be accounted for in this way.

3. THE EFFECT OF QUININE AND IMMUNE SERA ON EXOERYTHROCYTIC PARASITES

James and Tate (1938) have shown that quinine will cause an almost complete disappearance of parasites from the peripheral blood in infections with *P. gallinaceum*, but that the stages in the reticulo-endothelial system are not affected by the drug. This brings about a complicating factor in the effect of therapeutic chemicals on malarial infections which exhibit exoerythrocytic stages. Manwell and Goldstein (1939a) have similarly noted that immune sera from *P. circumflexum* infections, although very effective against the stages which occur in the erythrocytes, seems not to act as well on the parasites in monocytes.

4. THEORIES REGARDING THE NATURE OF EXOERYTHROCYTIC PARASITES

Various theories have been expressed with regard to the relationship between exoerythrocytic stages and other stages in the life cycle of bird malaria. In *P. elongatum* infections it seems quite probable that the penetration of reticulo-endothelial cells and white blood cells by merozoites, and their subsequent growth to maturity in these cells, represents an auxiliary cycle in the asexual development which is always present. James and Tate (1937, 1938), Brumpt (1937), Raffaele (1934a, 1936b), Corradetti (1938c), Giovannola (1938), Kikuth and Mudrow (1937, 1938, 1940), and Kikuth (1937b) believe that there is little doubt that the stages which they describe in *P. gallinaceum, P. relictum*, and *P.*

circumflexum infections are part of the life cycle of the malaria parasite in the vertebrate host, and represent a supplementary method of reproduction (plate XII). The fact that they may also be associated with the fate of sporozoites in the vertebrate host has already been referred to, and a recent paper by Kikuth and Mudrow (1940), which will be more fully discussed later, also expresses this view. Chortis (1938) considers the development of schizonts in the cells of the reticulo-endothelial system to be an accidental occurrence as a consequence of the reduced phagocytic function of the elements of the reticulum.

Hegner and Wolfson (1938a) have directed attention to the fact that blood parasites frequently found in birds and grouped under the name *Toxoplasma* resemble exoerythrocytic parasites found in connection with certain strains of avian plasmodia. This possibility is also considered by Manwell (1939). A brief description of organisms described as avian *Toxoplasma* is therefore given in order that the arguments for and against the theory that mixed infections may be involved in cases where exoerythrocytic schizonts occur may be understood better.

a. *Avian "Toxoplasma."* Wolfson (1940b) has recently discussed three types of organisms which have been described as avian *Toxoplasma*, some of which may be confused with exoerythrocytic bodies found in connection with malaria infections. This genus was established by Nicolle and Manceaux in 1909 for parasites which they found in the gondi, a small North African rodent. The organisms are infective to birds, frogs, and a number of species of mammals. They are intracellular parasites of small size, and occur characteristically in monocytes, although they have been found in other types of cells. *Toxoplasma* is ordinarily not commonly found in the peripheral blood, but inhabits the spleen, liver, bone marrow, kidneys, lungs, brain, mesenteries, and the body fluid. Reproduction is generally by binary fission. They may be round or crescent-shaped, and lack karyosomes.

Parasites which fit this description have been reported from birds, either under the name *Toxoplasma* or under

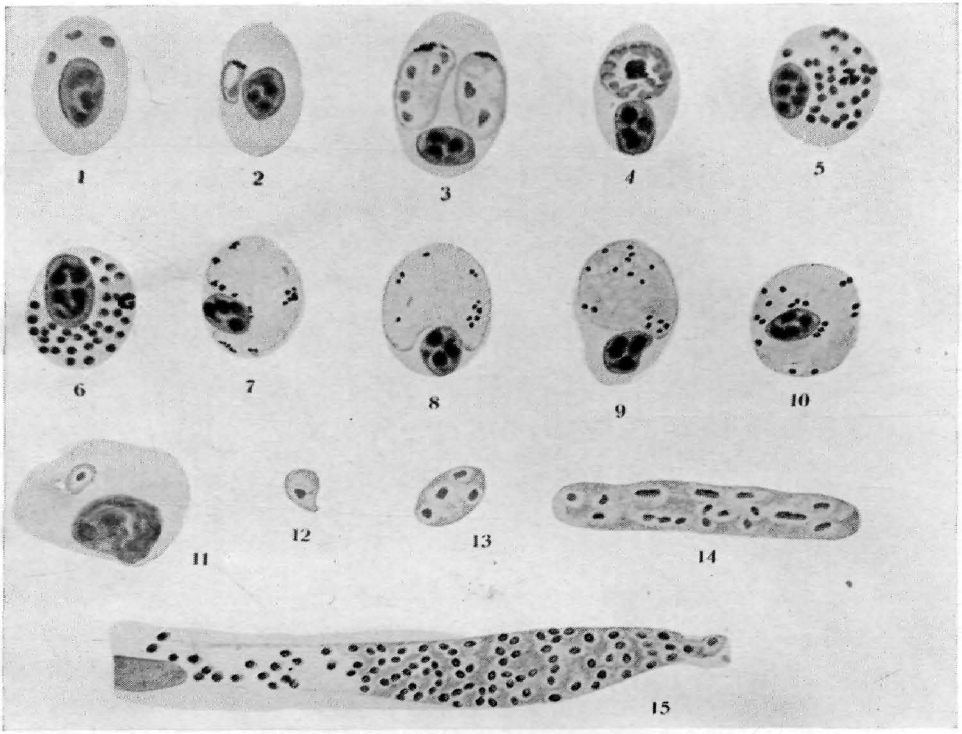

PLATE XII—Stages in the life cycle of *Plasmodium gallinaceum* (from Giovannola, 1938). (x1700).

1. Ring stages. 2. Young trophozoite. 3 and 4. Presegmentation forms. 5 and 6. Mature segmenters. 7 and 8. Male gametocytes. 9 and 10. Female gametocytes. 11. Uninucleate stage in a white cell. 12 to 15. Exoerythrocytic stages in brain capillaries.

some other generic name. Furthermore, various kinds of blood parasites which obviously do not belong to the genus have likewise been labelled *Toxoplasma*, so that the present status of nomenclature is greatly confused. The description by Laveran (1900) of intraleucocytic parasites of unknown nature, found in connection with a malarial infection in a Java sparrow, has already been referred to, and Herman (1937c) believes that this report represents the first description of *Toxoplasma* in birds. Adie found a similar parasite in sparrows in 1908. The parasites described by Laveran and Adie were named *Haemogregarina paddae* and *H. adiei* by Aragão (1911), who also described seven other species of this genus from Brazilian birds. An immediate controversy resulted, and Marullaz (1913), Hoare (1924), Carini and Maciel (1916), Nöller (1920), and Herman (1937c) believe that the parasites which Aragão saw were really *Toxoplasma.* In plate X (figures 1, 2 and 3) several of Aragão's drawings are reproduced; it seems quite clear that he was dealing with at least 2 and probably 3 different types of parasites. Whether or not all of these belong to the genus *Toxoplasma* is still disputable.

Herman (1937c) gives figures of several types of parasites which he found in sparrows. Among these are some of the parasites figured by Aragão, but in addition another type is described and illustrated, which appears to be the type of segmenting bodies claimed by James and Tate and others to be exoerythrocytic schizonts of malaria parasites. Herman gives the name *Toxoplasma paddae* to all of the organisms which he found in sparrows; some of his drawings are reproduced in plate X.

Other investigators have described some of the types of intraleucocytic parasites figured by Aragão, Herman and others from both wild birds (Wood and Wood, 1937; Hewitt, 1940b) and from experimental laboratory canaries (Wolfson, 1940b), but there is no general agreement as to what they really are. Wolfson gives a good summary of the literature and separates the organisms described by the above workers into three groups, calling them *Toxoplasma*-like

bodies. Under type I *Toxoplasma*-like bodies, all of the stages described as exoerythrocytic schizogony in bird malaria infections are grouped, these organisms representing the large schizogonic masses found in the endothelial cells of the brain capillaries, as well as the smaller schizonts in reticulo-endothelial cells of the spleen, liver, and bone marrow. As type II *Toxoplasma*-like bodies, she includes the oval, intraleucocytic parasites described by Laveran (1900), Aragao (1911, in part), Herman (1937c, in part), Wood and Wood (1937, in part), and Hewitt (1940b). This type is illustrated in plate X, figure 6. Type III *Toxoplasma*-like bodies are what she considers to be the "true" *Toxoplasma*, as described by Nicolle and Manceaux from mammals. These are shown in plate X, figure 8.

Manwell (1939) gives a comparison of *Toxoplasma* and exoerythrocytic bodies associated with malaria, and states that the most important difference between the two forms is the mode of multiplication, the former reproducing by binary fission and the latter by multiple fission. The greater specificity of the exoerythrocytic stages in malaria infections is another difference to which he attaches significance.

b. *Evidence for and against exoerythrocytic schizogony as part of the life cycle.* Kikuth and Mudrow (1937), Hegner and Wolfson (1938a), and Manwell (1939) have presented arguments for and against the theory that exoerythrocytic schizogony associated with bird malaria infections is part of the life cycle in these cases. The following points are among those which lead to the belief that these bodies are part of the life cycle:

a. Exoerythrocytic schizonts have been noted in various species of bird malaria, and in birds from widely different parts of the world.

b. Although attempts have been made to separate them from the stages occurring in the erythrocytes, this has not yet been done.

c. They occur in mosquito-induced infections as well as after direct blood inoculation.

d. Uniform failure has resulted in attempts to transmit them to animals which are known to be susceptible to mammalian *Toxoplasma.*

e. They are different from the " true " *Toxoplasma* which occurs in birds in that they reproduce by multiple fission.

f. Nothing resembling the exoerythrocytic stages which occur in the brain of malaria-infected birds have ever been reported from birds not infected with malaria.

Several other points in favor of exoerythrocytic stages as part of the life cycle of malaria are given by Hegner and Wolfson, and Manwell, but the above arguments are the most important.

The following points are in favor of mixed infections with *Plasmodium* and some other species of parasite:

a. Several laboratory strains of bird malaria have been carried through experimental birds over a period of years without showing exoerythrocytic stages. Some of these are strains of species which do exhibit these bodies.

b. In all species and strains of malaria in which they occur the exoerythrocytic stages appear to be identical, and show no morphological differences when they are associated with different strains and species of plasmodia.

c. They do not occur in all severe cases of infection with species generally associated with their presence, although their appearance is generally characteristically associated with heavy infections in the peripheral blood.

d. Inasmuch as malaria plasmodia show close morphological and physiological relationships in every other respect, it would be expected that all species and strains would show exoerythrocytic schizonts at one time or another if they are part of the life cycle. They have not yet been observed in monkey malaria nor in all species of bird malaria, and the few reports from human cases are of doubtful significance.

e. Certain other blood parasites exhibit stages which are

remarkably like the bodies in question, and it has not yet been conclusively proven that the segmenting forms of some of these other parasites are not involved in the present controversy.

c. *Attempts to separate Plasmodium from exoerythrocytic parasites.* Hegner and Wolfson (1938, 1939) tried several methods to separate *Plasmodium* from the exoerythrocytic parasites found in the same birds. Subinoculation, passage through mosquitoes, treatment with quinine, differential viability experiments, inoculation into chicks, centrifugation, and tissue culture were resorted to, but in no case were any of these successful.

B. The Fate of Sporozoites in the Vertebrate Host

In spite of Ross's observation that when sporozoites are introduced into a bird by the bite of a mosquito, infection results, the greatest gap in our knowledge of the life cycle of both avian and human malaria today is the fate of the sporozoites in the vertebrate host. Schaudinn (1902) seems to have been the only worker to observe the penetration of red cells by sporozoites (*P. vivax*) but his work is seriously questioned by most malariologists. The problem offers a fertile field for investigation in bird malaria, and a number of attempts have been made to discover what happens to sporozoites in the blood stream and visceral organs of birds.

Both Grassi (1901) and Ruge (1901) observed sporozoites moving about in suspensions of bird blood, but the penetration of red cells did not occur. The fact that sporozoites produce infection, however, when directly inoculated into the blood stream, as well as after mosquito inoculation has been amply confirmed (Wolfson, 1936b; Gambrell, 1937; Raffaele, 1937c; Warren and Coggeshall, 1937).

Raffaele (1937c) found that blood samples taken from canaries which had received intravenous inoculations with massive doses of sporozoites (*P. relictum*) are not infective to new canaries for many hours after inoculation. Furthermore, he points out that the prepatent period following

sporozoite injections is much longer than after direct blood transfer. His conclusion is that these facts exclude the possibility of a direct penetration into red corpuscles. Since he found exoerythrocytic parasites in the strain of *P. relictum* with which he was working, he believes that these parasites resulted from the penetration of sporozoites into reticuloendothelial cells. This theory, however, cannot be accepted without question, since the schizogonic cycle in tissue cells takes place both after blood inoculation and after the introduction of sporozoites, demonstrating that if exoerythrocytic parasites are part of the life cycle of malaria their occurrence may be independent of sporozoites (Corradetti, 1938e; James, 1939).

In a series of papers on sporozoites and sporozoite infection with *P. relictum,* Missiroli (1933, 1934, 1937, 1938) describes morphological changes in the sporozoites very soon after inoculation into birds. He pictures nuclear divisions in sporozoites (plate XIII, B) and their reproduction into a number of small parasites, and believes that what has been called the sporozoite is really a sporocyst, which after being introduced into the vertebrate host divides into a number of very small parasites and completes development in the lymph spaces, giving rise to not more than 8 true sporozoites.

Warren and Coggeshall (1937) infected canaries intravenously and intramuscularly with sporozoites of *P. cathemerium* but were unable to find parasites of any kind in the visceral organs or blood stream by direct observation until the usual asexual stages of this species became patent. When sporozoites were introduced directly into the blood stream, they were rapidly removed from the circulation in about 1 hour. After this interval they could not be demonstrated to be present by subinoculation for 72 hours. Blood withdrawn from canaries which received sporozoite injections 48 hours previously did not produce infections when inoculated into new birds, but emulsions of the spleen, liver, bone marrow, and pectoral muscle at the site of injection did prove infectious. Emulsions of the pectoral muscle at the site of injection produced infections in new canaries 1 hour, 24 hours, 48 hours, and 96 hours after sporozoites were introduced. This

demonstrates that parasites of some kind were present in the visceral organs, but probably not in the peripheral blood 48 hours after the introduction of sporozoites, but these could not be found either in direct smears or in fixed tissue preparations. The large doses of sporozoites used by these authors and the rapid clearance of sporozoites from the blood stream

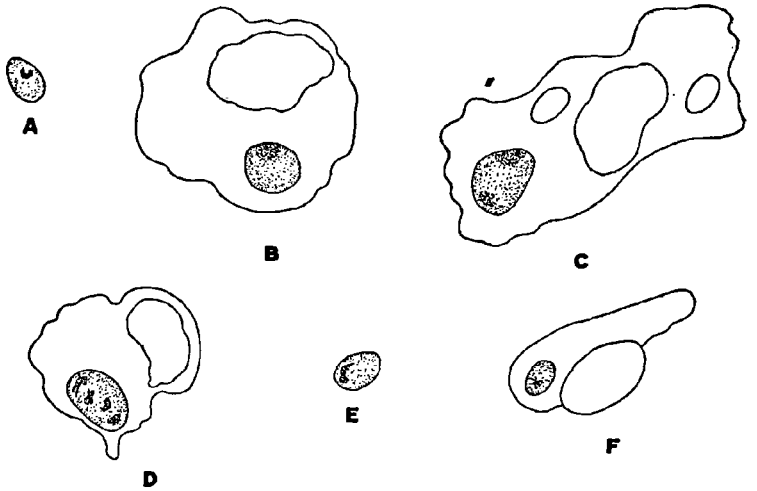

FIGURE 33—Line drawings illustrating one theory as to the fate of sporozoites at the site of injection (after Kikuth and Mudrow, 1940). (x3000).

A. *P. cathemerium*, free stage, 16 hours after injection.
B. *P. cathemerium*, uninucleate stage in a leucocyte, 40 hours after injection.
C. *P. cathemerium*, binucleate stage in a leucocyte, 48 hours after injection.
D. *P. cathemerium*, quadrinucleate stage in a leucocyte, 48 hours after injection.
E. *P. gallinaceum*, free stage, 56 hours after injection.
F. *P. gallinaceum*, uninucleate stage in a leucocyte, 56 hours after injection.

indicate a marked clearance mechanism on the part of the phagocytic elements of the blood. The fact that the presence of parasites could be demonstrated in the visceral organs by subinoculation to canaries within 48 hours after sporozoites were introduced, but not by direct microscopic observation indicates that either a small number survived phagocytosis, or their appearance was so changed within the visceral organs that they could not be recognized by the usual methods.

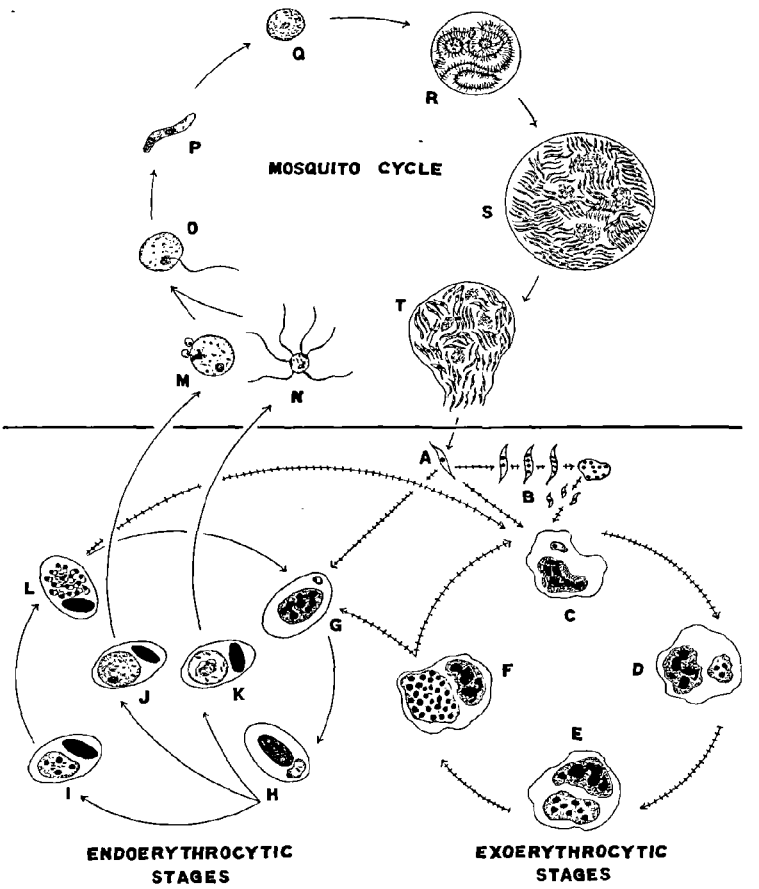

PLATE XIII—A schematic life cycle of bird malaria parasites, including certain stages which are not yet well understood. The barred lines indicate developments which have been suggested in recent work, as described in the text, but are not accepted by all investigators.

A. Uninuclente sporozoite. B. Nuclear division within sporozoites (after Missiroli, 1938). The larger forms are thought by Missiroli to be sporocysts, and the smaller division products, sporozoites. C, D, E, F. Growth stages within leucocytes. Raffaele (1937) and Kikuth and Mudrow (1940) believe these stages immediately follow the injection of sporozoites. G. Ring stage in a young red cell. H. Young trophozoite. I. Young schizont. J. Female gametocyte. K. Male gametocyte. L. Segmenter. M. Female gamete with two polar bodies. N. Exflagellation and formation of male gametes. O. Fertilization. P. Oökinete. Q. Young oöcyst. R. Sporozoites beginning to form within an oöcyst. S. Mature oöcyst. T. Escape of sporozoites from an oöcyst.

Kikuth and Mudrow (1940) have observed that from 16 to 72 hours after the intramuscular injection of sporozoites of *P. gallinaceum* and *P. cathemerium* into unparasitized birds, mononucleated and segmenting parasites occurred in monocytes at the site of the inoculations (figure 33). They postulate that only after one or more generations within the monocytes do certain "hemotropic" merozoites enter erythrocytes and start the cycle in red cells.

It is clear that further experiments are needed before the fate of sporozoites in the vertebrate host can be determined. The little knowledge available indicates that they probably do not initiate directly the familiar asexual cycle in the red blood cells. If they do penetrate red cells immediately the number which is able to do so is exceedingly small, else they could be demonstrated by subinoculations soon after they enter the host. Whether or not some change in their morphological appearance takes place cannot be definitely assured, although the work of Missiroli points in this direction. James (1939) shows that the time of appearance of exoerythrocytic parasites in *P. gallinaceum* infections is sooner after sporozoite inoculations than after direct blood transfer, and that the number of exoerythrocytic parasites is greater in infections produced by sporozoites. Coupled with the observations of Raffaele (1937c), Missiroli (1937) and Kikuth and Mudrow (1940) these facts lead to the belief that the reticulo-endothelial cells may represent the primary site of infection following the introduction of sporozoites into the vertebrate host, but this is not yet conclusively proven.

In plate XIII a schematic drawing of the life cycle of *P. cathemerium* is given, including certain developments which are not well understood at the present time. Where two or more lines lead from one stage to another, several possibilities are indicated, any one of which may represent the next stage in the cycle.

CHAPTER XI

PROBLEMS FOR INVESTIGATION

Within recent years the use of induced malaria for the treatment of general paresis in human patients has provided a method for conducting research on practical problems directly in the human host. Experiments on monkey *Plasmodium* have also furthered our knowledge of immune reactions and drug therapy in the field in general. The chief difficulties encountered in the use of these larger experimental hosts for research problems are the expense involved and the lack of facilities in many laboratories not connected with hospitals. With the recent discoveries of species of malaria parasites in chickens and pigeons, and the successful transfer of *P. cathemerium* and *P. relictum* to ducks, the need for a large experimental host is met, and there are still many problems which can be successfully investigated in the canary. The questions listed below suggest various types of problems in bird malaria research which, when solved, will greatly augment our knowledge of the bird parasites themselves, and will probably serve for a better understanding of similar problems in human malaria. This is by no means a complete list of unsolved problems in the field, and indicates that the work of the investigator has just begun.

A. SPECIES AND HOST RECORDS

1. What are the geographical limitations of the various species of avian *Plasmodium* now known?
2. What is the incidence of bird malaria in tropical countries, and what bird hosts in these countries are infected?
3. Are there any domesticated birds in any part of the world which show a high incidence of infection?
4. Will a study of host records and migratory habits add

to our knowledge of the origin and evolution of different species of bird malaria parasites?

5. Are there species of bird malaria which are confined in nature to but a single species of host?

6. How does the incidence of bird malaria in parts of the temperate climate as yet uninvestigated compare with known records?

7. Can other animals be experimentally infected with bird malaria?

8. Are there species of bird malaria other than those now known?

9. Are all of the species now generally recognized "good" species, or are they variants of other known species?

10. How accurate a test of specificity is complete or partial absence of cross-immunity?

11. Can the relationships between species be accurately determined through cross-immunity tests?

12. What morphological and physiological changes take place when species are inoculated into foreign hosts?

13. Are the above changes sufficiently great to account for the large number of species which have been described?

14. Can hybrid species be formed through the union of gametes of different species?

15. How constant are the limitations of variation within species?

16. Do races of each species exist?

17. What would happen if gametes of human and avian malaria were crossed?

18. Can mutations be produced by exposure to X-rays, heat, cold, etc.?

B. Cytology

19. How does the structure of malaria parasites in wet films differ from that in dry films?

20. Are chromosomes involved in the asexual division stages and in the formation of gametes?

21. Does a reduction of chromosomes occur in the maturation of gametocytes?

22. How are gametes formed so quickly from male gametocytes in drawn blood or in the stomach of the mosquito?

23. Are gametocytes formed from asexual forms, and if so do definite " sex cells " occur in each schizont?

24. How are sporozoites formed within oöcysts?

25. Does nuclear division regularly occur within the sporozoites of all species?

C. Characteristics of the Asexual Cycle

26. How do the asexual cycles of species not yet investigated in this respect compare with known species?

27. What determines periodicity?

28. Are periodic phenomena always exhibited in 24-hour cycles or in multiples of this figure, or do some species show 12 or 18-hour periodicity?

29. What is the reason for the reduction in the number of merozoites in mature schizonts near the peak of the parasite number?

30. Do the merozoites of all species penetrate young red cells?

31. Why do the merozoites of some species penetrate young red cells?

32. Why does the parasite number decrease at the time when young red cells are increasing?

33. Will the reduction of young red cells by artificial methods reduce the parasite number throughout infections?

34. Why are the segmenting stages of *P. elongatum* localized in the visceral organs?

35. Is exoerythrocytic schizogony part of the asexual cycle in bird malaria infections, and if so why is it not associated with all species?

D. Sexual Stages in the Vertebrate Host

36. Do the gametocytes of all species exhibit periodicity?

37. Is there an increase in the percentage of gametocytes formed at any time during the infection?

38. Are gametocytes localized to any extent in any part of the body?

39. What is the length of life of gametocytes?

40. Why are gametocytes not held in the visceral organs in *P. elongatum* infections?

41. What is the ratio of male to female gametocytes throughout infections?

42. What factors account for the above ratio?

43. What determines whether a gametocyte shall be elongate or round?

44. What happens to sporozoites in the vertebrate host?

E. Pathology

45. Is a toxin produced by bird malaria parasites?

46. Does the presence of parasites within the bird host elicit temperature changes of any sort?

47. What is the cause of death in bird malaria?

48. Are pathological lesions of any severity produced in visceral organs other than the spleen, liver and brain?

49. What changes take place in the blood plasma during heavy infections?

50. What pathology is exhibited in chickens, pigeons, and ducks infected with malaria?

51. Do infections in nature produce severe pathology and death?

F. Immunity

52. Is there any stage during the asexual cycle when parasites are destroyed by the host in greater numbers than at any other stage?

53. Is there an age resistance in bird malaria infections?

54. Does a weight resistance occur?

55. What is the nature of humeral antibodies in bird malaria infections?

56. Can tests be devised which will utilize these antibodies for serum diagnosis?

57. Can vaccination with killed parasites be successfully accomplished?

58. Can large doses of immune sera be used prophylactically?

59. What mechanism governs the resistance of some of the larger birds to infections with parasites isolated from smaller birds?

60. Is immunity in bird malaria gradually acquired, or is it developed suddenly?

61. Is immunity to superinfection absolute, in that no amount of infective blood will overcome it?

62. What is the mechanism of immunity in non-susceptible mosquitoes?

63. Is the tendency to relapse a genetic factor within strains?

64. Will malnutrition, exposure to heat or cold, etc. reduce resistance to bird malaria infections?

G. The Sexual Cycle in the Mosquito

65. How does the length of the mosquito cycle compare in the various species of bird *Plasmodium*?

66. Is there any method by which bird malaria sporozoites may be differentiated from those of human malaria?

67. Can bird malaria oöcysts be differentiated from those of human malaria?

68. What kinds of mosquitoes other than those now known are capable of transmitting bird malaria?

69. What effect does temperature and other external environmental influences have on the development of bird plasmodia in the mosquito?

70. How long will sporozoites remain viable in the mosquito?

71. How many sporozoites are produced within an oöcyst?

72. How many sporozoites are necessary to produce infection in the vertebrate host?

73. Do oöcysts and sporozoites of different species differ morphologically or physiologically?

H. Drug Therapy

74. Can a drug be found which will completely sterilize an infected bird?

75. Are there completely selective gametocidal drugs?

76. How do the drugs which are now used act on the parasites?

77. Can a chemical be found which will retard the normal asexual cycle?

78. Is there a drug which will act on the sporozoites and can therefore be used prophylactically?

79. Do certain drugs regularly produce relapse?

80. What stages in the life cycle are most vulnerable to drugs?

81. What effect will hormones have on the course of malarial infections in birds?

I. Miscellaneous

82. What conditions are necessary for the successful *in vitro* cultivation of bird malaria parasites for more than one asexual generation?

83. What explanation can be given for the changes in morphology and physiology of certain avian plasmodia when they are placed in abnormal hosts?

84. How are the avian plasmodia related to *Haemoproteus, Leucocytozoon,* and certain other genera in the evolutionary scale?

85. How do male and female gametocytes force their way out of host-cells when placed in isotonic saline or in the mosquito stomach?

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